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The Journal of Laboratory and Clinical Medicine

VOL. 20 JUNE, 1935 No. 9

CLINICAL AND EXPERIMENTAL

OBSERVATIONS ON THE AUTONOMIC CONTROL OF CARDIAC VASCULATURE*

ATTEMPTED VAGUS AND SYMPATHETIC OVERACTION ON CAT'S HEART

HAROLD F. ROBERTSON, M.D., AND ARTHUR J. DERBYSHIRE, A.B.,
BOSTON, MASS.

SEVERAL investigators have demonstrated that it is possible to anastomose a somatic motor nerve to an autonomic nerve and have the somatic fibers grow into the autonomic sheath.[1, 2] That such growth occurred was shown by physiologic responses in the organs connected with the affected innervation when it was stimulated, and after the death of the animal by histologic examination. In only one series of experiments however were spontaneous anatomic and physiologic changes produced. Cannon anastomosed the caudal end of the cut phrenic nerve of the cat to the cephalic end of the cut cervical sympathetic cord.[2] Changes developed similar to hyperthyroidism presumably by overaction of the sympathetic end-organs in the thyroid gland.

The object in our experiments was to test the ability of similar nerve anastomoses to activate appropriate end-organs when phrenic impulses and phrenic fibers could be shown conclusively to have extended toward the region of the involved end-organs or ganglia. To determine that the phrenic fibers had grown toward these regions we tested the nerves with modern electrical apparatus for the electrical phenomena that accompany their nerve impulses, and later checked these findings by histologic study. In the event of signs of activity developing we planned to produce at will certain changes in the organs affected and to alter

*From The Laboratory of Surgical Research and Laboratory of Physiology, Harvard Medical School.

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their activity by appropriate sutures. Because of our interest in cardiac circulation we chose the cardiac autonomic innervation as the field for experimentation. We hoped, at least, to cause tachycardia with vascular dilatation of cardiac vessels by phrenic-sympathetic anastomoses, and bradycardia with vasoconstriction of cardiac vessels by phrenic-vagus anastomoses.

Besides the evidence presented by Cannon there have been, from time to time, reports on the effects of sympathetic overstimulation produced however by means other than nerve anastomoses. Von Bechterew, among others, demonstrated that sympathetic irritation led to vascular spasm and endarteritic changes in the region supplied by the affected nerves.[3, 4]

Recently Adamson and Aird, by cutting the sacral outflow in cats, produced changes in the bowel similar to Hirschsprung's disease, the result apparently of unopposed sympathetic activity.[5] Though investigators have cut one or other, or both, the autonomic outflows to the heart with no noticeable pathologic change in the heart muscle, and no change in the vasculature, we repeated this procedure in some cats. We also, in some of the nerve suture experiments, cut the antagonistic nerve supply to the heart on the grounds that any effects of overstimulation would be amplified.

METHOD

Our preparations were as follows: In a series of cats unilateral or bilateral anastomosis was done between the cephalic end of the cut phrenic nerve, or one of its roots, and the caudal end of either the sectioned cervical sympathetic trunk or the sectioned vagus nerve or the cephalic end of the cut thoracic sympathetic chain. The opposing innervation to the heart was eliminated in a few cases. In both the case of the caudal end of the cut vagus nerve and the cephalic end of the cut thoracic sympathetic chain, most of the fibers are cut distal to their nuclei, and therefore degenerate since they are connector or preganglionic fibers with nuclei in the brain stem and cord respectively. This leaves room for ingrowing anastomosed fibers. In the case of the caudal end of the cut cervical sympathetic cord, however, the fibers are mostly postganglionic or effector with nuclei in the stellate ganglion and survive leaving, in theory, no opportunity for the ingrowth of phrenic fibers. Moreover it has been held that anastomoses should be made only to autonomic nerves containing a preponderance of preganglionic fibers such as the vagus nerve contains, rather than to nerves with a majority of postganglionic fibers as in the cervical sympathetic cord.[6] The nerve sutures were done with a fine eye needle and the finest silk thread. No suture was done under tension.

In other cats unilateral or bilateral division of either or both the sympathetic or parasympathetic innervation to the heart was carried out, the principles laid down by Cannon being followed with care so that all fibers to the heart were actually divided.[7]

The preparations thus secured were studied by taking frequent pulse counts, and watching cardiac reaction to fear, rage, atropine, and to asphyxia.[7] One might have expected, if the vagus end-organs in the heart were stimulated by anastomosis of the vagus to a rhythmically discharging nerve such as the phrenic,

that the heart would be slowed rhythmically or permanently and that fear and rage would have little speeding effect on the rate while atropine would allow the heart to speed up especially if adrenaline secretion were stimulated by fright or rage. Asphyxia from its earliest stages should greatly slow the heart but atropine should allow escape from this effect or prevent it. Conversely if the sympathetic end-organs of the heart were stimulated by phrenic impulses the heart should be more rapid than usual, fear and rage should increase the rate of beat and atropine should allow still further increase in rate. Asphyxia should greatly speed the heart, atropine perhaps accelerating the effect. In those cats where, besides nerve suture, denervation of the opposing system was carried out, inhibitory or stimulatory effects of the excised system on the heart's reaction to phrenic stimulation would of course be lacking. In the group that had the parasympathetic system, the sympathetic system or both systems removed reactions to fear or rage and atropine were useful in determining the presence or absence of fibers of either system. Whenever the chest was opened the heart was usually thoroughly inspected. The progress of the growing nerve anastomoses was followed by exposing the vagus or sympathetic nerves in either the neck or the chest and testing them for phrenic nerve impulses by a special technic which we describe under our observations.

OBSERVATIONS

To follow the progress of the anastomosed nerves along their new sheaths we used a voltage amplifier called the "Neurophone" (Fig. 1). It was possible by this means to detect phrenic nerve impulses without disturbing the nerve or its sheaths. The neurophone consists of four stages of transformer coupled amplification that are run by batteries contained in the same steel case. The output of this amplifier feeds two more stages of amplification and a loud-speaker in a separate steel case for audibly detecting the responses. This power stage can run on either alternating current or direct current. These two portable units were built by Mr. E. L. Garceau, in the Laboratory of Physiology, for use in operating rooms to examine the action potentials of nerves and muscles.[8] The amplifier can detect inputs of about three microvolts.

For the particular purpose of recording the action potentials of the phrenic nerve or the potentials of phrenic fibers that had regenerated into the sheath of either the sympathetic or the vagus nerves in the neck or chest, it was necessary to combine high sensitivity with good shielding. Sensitivity was necessary in order that impulses might be recorded from fibers with small diameters, such as those of regenerated nerves, since the action potential is proportional to the cross-section of the nerve fiber, and in order that the sheath around the nerve would not have to be removed or disturbed with consequent interference with the regeneration of the nerve fibers.[9] From a series of acute preparations of the cat's phrenic nerve it was found that the farther apart the ground lead and the differentiated lead were placed, up to 1 cm., the better were the responses obtained. In the chest it was found that the E. K. G. was the main source of interference and an adequate shield from this disturbance had to be provided. In the neck as well as in the chest the spread of the action potentials of the muscles of

respiration was a confusing component that had to be completely eliminated if definite results were to be obtained.

We were finally able to avoid these forms of interference and still obtain enough sensitivity by the use of a special type of electrode suggested to us by Dr. Hallowell Davis (Fig. 2, *1*). This consisted of a thin silver plate about 6 mm. wide that was bent in the shape of the letter J. The inner side of the curve was insulated with a baking varnish, and a piece of silver wire placed on this varnished surface was baked in place. The silver wire was connected by an adapter to the grid of the first tube of the neurophone through a sterilizable shielded cable. The shield of this cable was continuous with the flat silver plate of the electrode and was led to the ground of the amplifier. The baking varnish was scraped

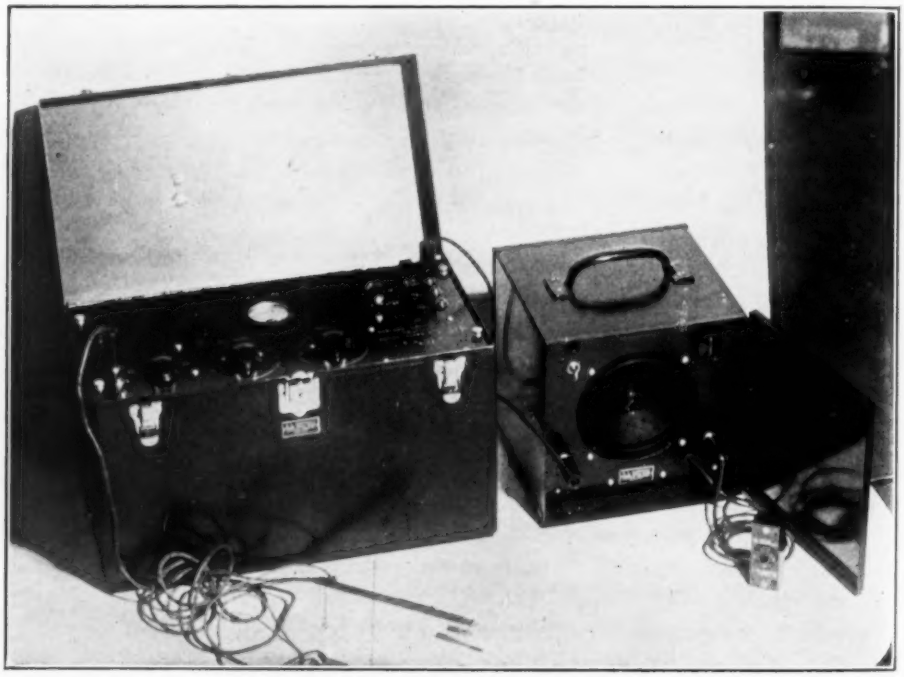

Fig. 1.—The neurophone. Input and amplifying circuit, left; loud-speaker, right; sterilizable cable and electrodes in foreground.

away from the outer edges of the bent silver plate and from the silver wire in the center. The electrodes thus prepared were sterilizable. The exposed nerve with its sheath intact could be placed upon this electrode without interference with either its sheath or its connections. The nerve made three contacts; the first one to the silver plate; the second to the differentiated lead to the grid of the first tube; and the last to the silver plate again. Extraneous electrical disturbances which travel along the surface of nerves, such as the E. K. G. and the spread of the action potentials originating in the respiratory muscles, were shunted through this ground plate and not recorded. The potentials developed by the nerve fibers, however, were recorded as differences of potential between the silver wire in the middle of the electrode and the points of the nerve touching the silver plate.

The proof that this electrode was reliably shielded depended upon many cases in the normal cat in which nothing was obtained from the normal sympathetic nerves in the neighborhood nor from a strip of connective tissue that was left connected to the animal body, at both ends, and yet the phrenic nerve gave audible responses correlated with respiration. Moreover similar tests were carried out in the cats in which the phrenic nerve had been anastomosed to the sympathetic or vagus nerve. In these too we found that extraneous impulses were not reproduced by our amplifier. The fact that impulses, synchronous with respiratory and cardiac rhythms, have been demonstrated in sympathetic and vagus fibers coursing toward the heart was kept in mind.[10] In several acute experiments on cats we found that the neurophone did not pick up such impulses though at the same time the characteristic normal phrenic impulses were heard very well. Impulses were also detected in the normal vagus nerve during the inspiratory phase of artificial respiration especially if excess inflation occurred. The same impulses were not heard during spontaneous respiration. They

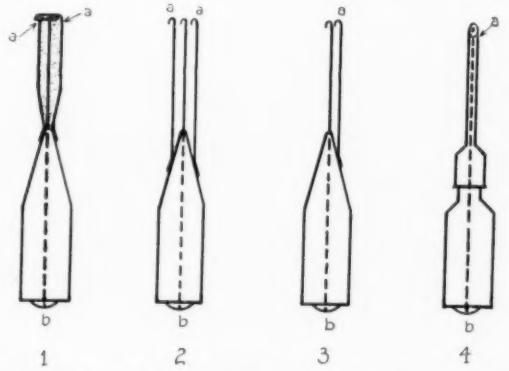

Fig. 2.—Electrodes used with the neurophone. a, Ground lead; b, differentiated lead (dotted line). *1*, Moderate sensitivity, excellent shielding and selectivity. This electrode was found best for our purpose. *2*, Very sensitive, good shielding and selectivity, but hard to place on the nerve. *3*, Fairly sensitive, poor shielding. *4*, Shielding and selectivity good, but needle could not be forced into small nerves well.

were interpreted as afferent vagus impulses arising from the lung and respiratory passages. We were certain that impulses other than those of the phrenic nerve, but perhaps capable of resembling them, would not confuse our observations. In numerous instances, when typical phrenic impulses were found, both above and below the level of the nerve suture in such a preparation, histologic examination confirmed the presence of healthy nerve fibers growing in the anastomosed nerve sheaths.

We tried other forms of electrodes but for various reasons they were found inadequate. These electrodes are all shown in Fig. 2. The electrode shown in Fig. 2, *2* was found to be sensitive and to shield out the extraneous activity but the wires were too flexible and were hard to place under delicate nerves. The electrode in Fig. 2, *3* lacked good shielding but was good in sensitivity. Due to the amount of E. K. G. and muscle action potentials picked up this electrode was discarded. Finally the fourth type gave so much selectivity and shielding that the phenomena of the nerve could not be detected. This electrode could

only be used if it was introduced into the nerve through the sheath. This produced so much damage that it was not serviceable.

We can summarize our technical procedure by describing the limitations of the method. The noise level of the amplifier at about 2 μ determined the limit of the overall sensitivity that could be obtained. The potential changes detected from regenerated nerves are small because first, the potential developed is directly proportional to the cross-section of each fiber, and second, the sheath of the nerve shunts much of the potential change. These conditions require sensitivity of detection. Finally we could not use as great a separation of ground and differentiated leads as desirable since it was not always convenient to expose the required length of nerve. The method is not as sensitive as desired, but for the present we are content with being certain that such responses as we have obtained are definitely those of the nerve fibers in question.

With such a technic we have little difficulty in deciding whether certain portions of nerve sheath contained phrenic impulses. Six animals in which nerve suture was done gave positive evidence of successful anastomoses. Care had to be taken during tests that the nerve was not subjected to tension and that the sheath was not traumatized, otherwise the nerve might be thrown into a state of temporary block and no result obtained. In several animals with phrenic nerve anastomosed to vagus, normal phrenic impulses were picked up as far caudally as the aortic arch, and in one cat with a phrenic sympathetic suture phrenic impulses were followed along the cardiac fibers from the stellate ganglion toward the heart. Histologic section as mentioned above confirmed electrical findings in many instances.

Though some of the cats have now lived for over a year with successful nerve anastomoses no gross anatomic change has taken place.* In numerous autopsies on cats with either successful nerve sutures or on cats whose cardiac innervation had been interfered with by some other method no histologic change was noted.

In the cats with nerve anastomoses and in those with section of some part of the cardiac innervation there was no physiologic evidence that sympathetic or parasympathetic end-organs were overactive. Fear or rage, and atropine, in the case of the simple denervation experiments produced no unusual reactions other than those already outlined by Cannon and others as seen when vagus, sympathetic or complete cardiac denervation is done.[7] In the cats with nerve anastomoses alone or anastomoses in conjunction with denervation of the opposing system no evidence of sympathetic or parasympathetic overaction was found by the production of fear or rage, asphyxia, or the use of atropine, and so, although phrenic fibers had undoubtedly grown into the vagus and sympathetic sheaths, no effect on the respective end-organs was discovered by the physiologic tests employed.

*Two cats with successful phrenic-vagus anastomoses and one cat with successful phrenic-sympathetic anastomosis have (at time of publication) now been observed for over two years. In all three phrenic impulses could be traced electrically as far caudally as the aortic arch along the anastomosed vagus or cardiac sympathetic nerve sheaths. There is no evidence of anatomic or physiologic change in the hearts of these animals.

SUMMARY

In cats anastomoses between the phrenic and the vagus or the sympathetic nerves were done unilaterally or bilaterally and by electrical tests active phrenic fibers were shown to have grown past the point of anastomosis toward the heart. The electrical findings were confirmed by histologic studies. Though no effects on appropriate end-organs and associated viscera have been demonstrated, it must be remembered that in previous reports the commencement of such effects was often delayed well over a year. Therefore such cats as we have left are being carefully followed in the hope that later on effects will be established.* Our ineffectual efforts to create anatomic changes in the heart and its vessels by section of the sympathetic or parasympathetic cardiac fibers in an attempt to produce autonomic imbalance, correspond with previous observations in similar procedures on the heart. The technic of testing our nerve preparations provided a good example of what may be done in the field of neurosurgery with modern electrical apparatus; it is likely in the future to undergo still further development to the advantage of those who wish to define nerve tracts and study their activity.

We wish to thank Dr. Hallowell Davis whose suggestions concerning the electrodes and the amplifier have been indispensable.

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*See footnote on page 900.

THE VALUE OF THE TAKATA AND ARA REACTION AS A DIAGNOSTIC AND PROGNOSTIC AID IN CIRRHOSIS OF THE LIVER*

ALEX B. RAGINS, M.D., CHICAGO, ILL.

INTRODUCTION

THE mercury sublimate, diamond fuchsin test was first introduced in 1925 by Takata[1] as a laboratory test for differentiation between lobar pneumonia and bronchopneumonia. That same year Takata and Ara,[2] working on spinal fluid, introduced a modification of the test, thus affording a differentiation between the syphilitic and meningitic types of spinal fluids. Many German investigators[3] utilized the test for the determination of syphilitic meningitis with fairly good results. In this country, Monias,[4] in 1928, introduced the Takata and Ara test, commenting favorably as to its accuracy and simplicity.

Staub[5] mentioned the application of this test as an aid in the diagnosis of liver cirrhosis. In 1929, Jezler[6] utilized this test on blood serum and found that it would produce a flocculation in a great majority of instances where patients were suffering from cirrhosis of the liver. Jezler[7] again successfully carried out the test but with more detail and with some modification. Other investigators, Oliva and Pescoroma,[8] Skouge,[9] Crane,[10] and Hugonot and Sohie,[11] tried it with convincing findings.

METHOD

During the last year I have tried the test on 276 patients, using the Jezler modification which is as follows. The reagents required are a 0.9 per cent solution of sodium chloride, a 10 per cent solution of sodium carbonate, a 0.5 per cent solution of mercuric chloride, and a 0.02 per cent solution of diamond fuchsin. All these solutions are made with double distilled water.

A series of eight Wassermann tubes of the same diameter, rinsed in double distilled water and carefully dried, and each containing 1 c.c. of a 0.9 per cent saline solution, are set up. Then 1 c.c. of blood serum (nonhemolyzed and not more than twenty-four hours old) is added to the first tube. After the contents of this tube are well mixed 1 c.c. is pipetted off and placed in the second tube. Then 1 c.c. is withdrawn from the second tube and placed into the third. This procedure is repeated with the remaining tubes, and the last cubic centimeter is discarded. The final set-up then consists of eight tubes containing serum in the dilutions from 1:2 to 1:256. To each of the tubes 0.25 c.c. of a 10 per cent solution of sodium carbonate is added, and the contents are well shaken. Immediately afterward 0.3 c.c. of a freshly prepared Takata and Ara reagent,

*From the Department of Pathology, Cook County Hospital, Dr. R. H. Jaffé, Director.
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containing equal parts of a 0.5 per cent mercuric chloride and 0.02 per cent solution of diamond fuchsin, are added to each tube, and the rack is shaken thoroughly. This completes the procedure.

Readings are made immediately after, then one-half hour after, and twelve to twenty-four hours after the completion of the test. A positive Takata and Ara reaction is characterized by a flocculation occurring in the tubes having a dilution from 1:16 through 1:64; that is, flocculation must occur in at least these three tubes in order to have this considered a positive test. Jezler[7] claims that the strength of the reaction occurs at the time of flocculation. A three-plus T. A. R.* is present when flocculation occurs immediately, a two-plus T. A. R. is present when flocculation occurs one-half hour after the test is carried out, and a one-plus T. A. R. presents itself when flocculation occurs twelve to twenty-four hours after completion of the test. Plus-minus reactions are considered when questionable flocculations occur after twenty-four hours. In my series of cases, however, I found flocculation occurring immediately after completion of

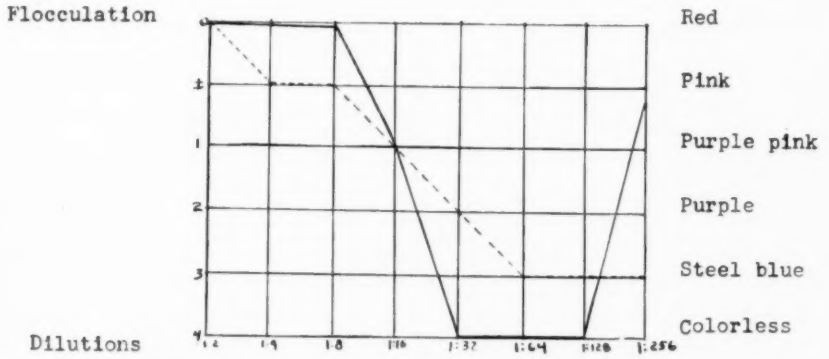

Fig. 1.—Dotted line shows color changes in a normal serum. Solid line shows flocculation curve in a positive T. A. R. serum.

the test in all my positive cases, with very little, if any, change in the flocculation after twelve to twenty-four hours, and I was led, therefore, to classify the strength of the T. A. R. on the basis of four-plus, three-plus, two-plus, one-plus, plus-minus, and negative, depending upon the heaviness and amount of the flocculations that occurred.

The colors produced in a series of test tubes, especially in the negative T. A. R., are invariably constant. The first tube has a red to a reddish pink color. The second and third tubes are pink; the fourth, purplish pink; the fifth, purple; and the sixth, seventh, and eighth tubes, steel blue (see Fig. 1).

Nicole[12] has shown that blood serums will persistently give this curve, and even in spinal fluid where blood serum is present such a curve will manifest itself. This curve, according to Nicole, is due to the albumin concentration in the serum.

RESULTS

In this series of 276 cases, the clinical diagnosis was used as a basis for classification of the diseases and wherever possible was substantiated by post-

*T. A. R., the abbreviated form of Takata and Ara reaction.

mortem examination or surgical biopsy. The cases were classified into fourteen main groups (Table I), with cirrhosis of the liver heading the list.

Of fifty-nine cases* diagnosed clinically as cirrhosis of the liver, forty-five cases gave a four-plus T. A. R. in the blood serum, four cases gave a three-plus T. A. R., three cases a two-plus T. A. R., and two cases a one-plus T. A. R. Three cases gave a plus-minus T. A. R. and two were negative.

In this group twelve of the cases were confirmed by postmortem examination or surgical biopsy of the liver. In one case, with a four-plus positive T. A. R., an exploratory operation of the abdomen was performed, and a large quantity of serosanguineous fluid was found. The liver, spleen, and kidneys were found to be grossly normal. Unfortunately, a biopsy of the liver was not taken, thus making impossible absolute proof of a cirrhotic process of the liver in this patient. In two of our autopsied cases primary carcinoma of the liver was found to be associated with cirrhosis of the liver.

In some cases of cirrhosis of the liver additional T. A. R. tests were carried out in ascitic fluid and pericardial fluid. With one exception, these were found to be positive. Ten cases of ascitic fluid were tested, nine of which gave a positive T. A. R. and one a negative T. A. R. One of pleural fluid gave a positive T. A. R.

As to the number of cases in the cirrhosis series with a positive T. A. R. that showed evidence of hepatic decompensation, 46 are enumerated in Table I. One case had ascites with a negative T. A. R.

Chronic consumption of alcohol was admitted in 43 cases with a positive reaction and in two patients with a negative reaction. Of the remaining thirteen cases a history of alcoholism was either not obtained or denied.

As to the mortality rate, 32 patients who had had a positive T. A. R. had died during the course of observation, a period of ten months. Two positive T. A. R. cases were sent home, and the eight remaining cases of the series are still in the hospital at this writing.

Other tests were run wherever possible, but although not in any manner complete, are still sufficient to compare with the T. A. R. Of all tests in cases of liver cirrhosis, the icteric index showed a marked parallelism with the T. A. R. Thirty positive cases showed an increased icteric index; four positive cases were normal. One negative case showed a high icteric index. As to the urea nitrogen of the blood serum in these cases, twelve positive T. A. R. cases showed an increase, and nineteen positive cases were normal, thus denoting that urea nitrogen is not a valuable adjunct in indicating liver damage. Of the two negative cases one showed an increase in urea nitrogen, and one was normal.

Of the fourteen cases of acute hepatic insufficiency other than cirrhosis of the liver, there were three cases with a three-plus positive T. A. R., and two cases with a plus-minus positive T. A. R. All these five patients improved and were sent home. Two of the three-plus cases were patients suffering from acute hepatitis and one from acute yellow atrophy. The two plus-minus positive cases were patients with acute hepatitis.

*In five cases T. A. R. tests were carried out on postmortem serum, and the reactions were no different from that of the serum of living patients. Skouge[9] has shown that tests in postmortem serum can be successfully carried out, provided the blood is not hemolyzed and the serum does not contain too much fat.

TABLE I

CLINICAL DIAGNOSIS	NO. OF CASES	T.A.R. OF BLOOD SERUM AND ASCITIC* AND PLEURAL† FLUIDS		P. M. OR SURGICAL FIND. IN POS. T.A.R.		P. M. OR SURGICAL FIND. IN NEG. T.A.R.		HISTORY OF ALCOHOLISM		ASCITES		UREA NITROGEN IN POSITIVE T.A.R.		UREA NITROGEN IN NEGATIVE T.A.R.		ICTERIC INDEX IN POS. T.A.R.		ICTERIC INDEX IN NEG. T.A.R.	
		POSITIVE	NEGATIVE	CONF. DIAG.	DIF. DIAG	CONF. DIAG.	DIF. DIAG.	POS. T.A.R.	NEG. T.A.R.	POS. T.A.R.	NEG. T.A.R.	INCREASED	NORMAL	INCREASED	NORMAL	NORMAL	INCREASED	NORMAL	INCREASED
Cirrhosis of liver	59	57	2	12	1			44	2	46	1	12	19	1	1	4	30		1
Acute hepatic insufficiency other than cirrhosis of the liver	14	5	9			1		1	5			2		2	1		3		8
Hepatitis due to arsenical and industrial poisonings	3	1	2						1				1		1		1		2
Malignant tumors without liver metastases	7	3	4	2		2		2	1	1	1	1		3	1		1	1	2
Malignant tumors with liver metastases and primary tumors of livers	20	11	9	1		5		4	7	5	2	3	2	7	3	3	2		5
Normal pregnancies	10		10																
Toxemias of pregnancy	6	3	3			1								1					
Cardiovascular renal disease other than syphilis	18	2	16			1		1	7	1	4	1		7	3			2	3
Diseases of the kidney	11	1	10			2			3		3			9	1			2	1
Acute, subacute, and chronic infections	54	12	42			3		7	7	2	4	4		8	7				2
Blood dyscrasias	10	1	9	1		2					1			2					1
Diseases of the glands of internal secretion	16	8	8			2			1	1			4	3			1		
Syphilis	4	1	3	1					1				1			1			1
Varia	44	9	35		1	7		6	10	1		2		3	3		2	3	3
Total	276	114	162	17	2	26		64	45	57	16	25	27	46	21	8	40	8	29

*Four cases of ascitic fluid tested.

†One case of pleural fluid tested.

Skouge[9] speaks of several cases of acute hepatic insufficiency which had positive T. A. R.'s during the active stage, and these positive reactions were negative when their conditions improved. The remaining nine cases were those of acute hepatitis, and all were negative. One of the two negative cases that died was autopsied. This case also gave a negative T. A. R. on the postmortem blood serum. At autopsy there was evidence of a severe acute hepatitis.

In eleven cases of this series, the icterus index was high, and of the remaining three no laboratory data were given.

In three cases of liver damage due to arsenical or industrial poisonings, only one case gave a strongly positive reaction. This man was being treated for syphilis two years after the infection, and following the first injection of arsphenamine, he developed a diffuse erythema, which was at first diagnosed as scarlet fever, but the erythema was soon replaced by a marked jaundice and coma. At that time his T. A. R. was a decided four-plus. At a later date, it was still a strong four-plus. The patient has had several relapses associated with small gastric hemorrhages and is at present in a poor state, despite the fact that he has released himself from the hospital.

There were seven cases of malignant tumors without metastases to the liver, three of which showed a positive T. A. R. Of these three positive cases, there was one four-plus T. A. R. in a carcinoma of the hepatic duct, associated with an obstructive biliary cirrhosis. This patient, a Japanese, gave a history of drinking a quart of alcohol almost daily for forty years. The second case was a carcinoma of the stomach, and the third manifested itself as a carcinoma of the prostate and was proved to be so by surgical biopsy. The patient died soon after operation, and a postmortem examination was not permitted. The remaining four cases were negative and consisted of one case of carcinoma of the stomach, one a carcinoma of the rectum, a third was diagnosed upon laparotomy as a carcinoma of the head of the pancreas and the fourth case was a carcinoma of the common duct, with marked evidence of biliary obstruction, as was pointed out at autopsy.

In the group of carcinomas with metastases to the liver and primary carcinomas of the liver, there is a total of twenty cases. Eleven cases showed evidence of liver damage, as indicated by a positive T. A. R. In the latter group, I found two carcinomas of the stomach to have a four-plus T. A. R. Two had a two-plus T. A. R., and two had a one-plus T. A. R. Three had a negative T. A. R.

Of the carcinomas of the head of the pancreas, the T. A. R. was negative in the only two cases which I had. In carcinoma of the extrabiliary tract, excluding carcinoma of the gallbladder, two cases were negative and one gave a plus-minus reaction, the latter test being run on the ascitic fluid.

Of three cases diagnosed as primary carcinoma of the liver, two were three-plus, and one was negative, the latter one proving to be a carcinoma of the liver, upon surgical biopsy of both the liver and a mesenteric lymph node.

In one case of neurocytoma with a negative T. A. R., the clinical diagnosis was substantiated by postmortem examination. In another case, in which the primary tumor was undiagnosed, there was a marked obstruction of the inferior vena cava. The T. A. R. on the serum was negative. In this group, the clinical diagnosis of six cases was confirmed by postmortem examination or surgery.

In a series of ten normal pregnancies, the T. A. R. was found to be negative in all cases. They subsequently delivered normally without any complications.

Six cases of toxemia of pregnancy were studied. In this series there were three cases which showed evidence of a positive T. A. R. In one case of pregnancy associated with hypertension the T. A. R. was two-plus, and in two cases of eclampsia the T. A. R. was one-plus. All three patients improved under proper management and left the hospital in better health than upon admission.

Of the remaining three cases, one, an eclamptic, gave a negative T. A. R. and at postmortem examination gave the findings typical of eclampsia. The other two cases which gave a negative T. A. R. were pregnancies associated with hypertension.

In a group of cardiovascular renal diseases other than syphilis eighteen cases were studied, of which only two cases gave a positive T. A. R. One was a case of cerebral hemorrhage and gave a four-plus reaction. The other was a case of arteriosclerotic heart disease, giving a one-plus T. A. R. Of the remaining sixteen cases, two were hemiplegias, one of which at postmortem examination showed the essential findings of a ruptured aneurysm of the anterior communicating artery. As an incidental finding there was an ecchinococcus simplex cyst in the right lobe of the liver. Four patients suffered from arteriosclerotic heart disease, nine patients were victims of hypertensive heart disease, and one case was considered a primary myofibrosis. All these heart patients were in a state of cardiac decompensation upon admission to the hospital, and when the test was carried out on them, four patients had marked ascites, and the others had edema of the lower extremities associated with dyspnea.

Of eleven cases classified as diseases of the kidney, only one case gave a plus-minus T. A. R., whereas all the others were negative. The single positive case was that of a bilateral hydronephrosis. The others consisted of two cases of chronic glomerulonephritis, one of a unilateral polycystic kidney, one of lipoid nephrosis, one of pyelonephritis, and five of malignant nephrosclerosis. The clinical diagnoses of two of these cases were corroborated at autopsy.

Of 54 cases classified under acute, subacute, and chronic infections, four cases gave a four-plus positive T. A. R. Two patients were suffering from acute rheumatic heart disease, both dying later. A third case was a far-advanced pulmonary tuberculosis and the patient had a history of drinking one quart of alcohol daily for five years. In the fourth case, the patient came into the hospital with lobar pneumonia, and after a stormy course recovered. Upon his recovery, it was found that he was an habitual drunkard and moderately demented.

One of the three cases having a three-plus T. A. R. was an acute rheumatic heart patient who later improved and went home. The second was a case of lobar pneumonia, and the third was a case of tuberculous pleurisy with effusion. The patient gave a history of drinking at least one pint of alcohol daily for the last sixteen years.

A one-plus T. A. R. was obtained in a case of pulmonary tuberculosis and in a case of lobar pneumonia.

In this series of infectious diseases, eleven cases of pulmonary tuberculosis, one case of gonorrheal arthritis, two of malignant endocarditis, five of bronchopneumonia, one of acute follicular tonsillitis, one of typhoid fever, one of acute articular rheumatism, twelve of lobar pneumonia, one of tuberculous polyserositis, one case of septic sore throat, one of postpneumonic empyema, one of tuberculous pleurisy with effusion, and one of septic meningitis, gave negative T. A. R.'s.

In ten cases of blood dyscrasia, only one case gave a positive T. A. R., the reaction being two-plus. This was a case of acute stem cell leucemia in an aleucemic state, associated with a septicemia due to a hemolytic streptococcus. The liver in this case showed, microscopically, a dense infiltration of undifferentiated cells in the portobiliary septa, as well as in the capillaries, and in addition to these, the liver cells were slightly displaced by intralobular cell accumulations.

The nine remaining cases of blood dyscrasia were negative. One was a case of acute stem cell leucemia, four were that of pernicious anemia, one an aplastic anemia, two were splenic anemias, and one was a case of aleucemic myelosis. In two instances postmortem examination confirmed the diagnoses.

As to the diseases of the glands of internal secretion and the response of their serum to the T. A. R., sixteen cases were studied. Eight cases showed a positive T. A. R. Two cases of exophthalmic goiter and one case of toxic adenoma gave a four-plus T. A. R. One case of toxic adenoma was a three-plus T. A. R., and one case of myxedema associated with a pituitary disturbance, as indicated by clinical evidence of acromegaly, gave a two-plus T. A. R. Another case of myxedema gave a one-plus T. A. R., and two cases of exophthalmic goiter gave a one-plus T. A. R. Of the remaining eight cases, seven exopthalmic goiters were negative, and one toxic adenoma was negative.

Four cases of syphilis were studied. All patients were negative except one, who had a very much enlarged liver, and upon laparotomy and biopsy of the liver, a diagnosis of syphilitic hepatitis was established. The reduction of liver parenchyma was striking. This patient's T. A. R. was three-plus positive. The other three cases were syphilitic aortitis, aneurysm of the aorta, and tabes dorsalis, respectively.

The last group under the heading of varia consists of a number of cases which are of various illnesses, each type having too few in number to be classified individually.

In this group four cases gave a four-plus T. A. R. One was a case of carbon monoxide poisoning, and upon being revived, the patient admitted alcoholism. A second case was that of delirium tremens; a third was given a tentative diagnosis of chronic malaria with a tremendously enlarged liver and spleen; and the fourth case was one of acute cholangitis.

A two-plus T. A. R. was found in a case of impending delirium tremens. In one case of bleeding duodenal ulcer and in another case diagnosed as Banti's disease, the T. A. R. was one-plus positive. Two cases gave a plus-minus reaction, one being a case of acute cholangitis with obstruction of the common duct, and the other a Picks' polyserositis.

The negative T. A. R.'s were found in four cases without objective clinical findings, three cases of common duct obstruction, one compression of the spinal cord, one brain tumor, two retroverted uteri, five cases of chronic cholecystitis, two of arteriosclerotic dementia, three of chronic alcoholism, three of alcoholic coma, one of peptic ulcer, two of malaria, one of acute hydrops of the gall-bladder, one of amytal poisoning, one of tumor of the right groin (fibroma), one of benign adenoma of the thyroid, one of dysmenorrhea, one of prematurity, one of schizophrenia, and one of acute intestinal obstruction.

In this group, the diagnoses of seven negative T. A. R. cases were confirmed by either postmortem examination or surgery, and one four-plus positive case, diagnosed as a common duct stone, upon laparotomy revealed a stenosis or stricture of the left hepatic duct.

DISCUSSION

In summarizing the total number of 276 cases, I find that 114 of those tested gave a positive Takata-Ara reaction varying from a four-plus to a plus-minus positive reaction. The 114 positive reactions were distributed among the different diseases as follows:

Cirrhosis of the liver	50.00%
Acute hepatic insufficiency	4.38%
Arsenical and industrial poisoning	0.87%
Malignant tumors with liver metastases	9.51%
Malignant tumors without liver metastases	2.63%
Normal pregnancies	0.00%
Toxemias of pregnancy	2.63%
Cardiovascular renal disease other than syphilis	1.75%
Diseases of the kidney	0.87%
Acute, subacute, and chronic infections	10.52%
Blood dyscrasias	0.87%
Diseases of the glands of internal secretion	7.01%
Syphilis	0.87%
Varia	7.89%

It is interesting to note that cirrhosis of the liver may be involved in cases of Graves' disease, as has been shown by Rössle,[13] Weller,[14] Assman,[15] and others. Raab and Terplan[16] have reported a case in which subacute yellow atrophy of the liver was a complication of exophthalmic goiter. Weller[14] states that the liver may be, and frequently is, involved in Graves' disease, as shown, clinically, by some occurrences of icterus, marked degrees of which are known to be of serious importance in this disease; physiologically, by the accumulating evidence of altered liver function in such patients; experimentally, by the evidence of hepatic dysfunction following administration of thyroid substance and thyroxin, and morphologically, by structural changes in the liver, varying from slight degrees of chronic hepatitis to a widespread degenerative and necrotizing process which must be considered an acute yellow atrophy.

In Weller's series of 48 cases of Graves' disease, well-marked hepatitis was found in 54 per cent of the cases, while a matched control series of the same size yielded but a single case with a well-marked hepatitis. Youmans and Warfield,[17] in their study, have found that exactly 50 per cent of their cases of hyperthyroidism showed evidence of liver damage.

In reviewing the cases of hyperthyroidism that came to autopsy in the last five years at Cook County Hospital, with the exception of two cases, which were posted and which gave a negative T. A. R. and no marked morphologic changes of the liver, I found that in nineteen cases posted 2, or 10.5 per cent, of the patients had jaundice associated with symptoms of hyperthyroidism, and one patient, or 5.2 per cent, had a peripheral type of cirrhosis of the liver, which is one of the types of cirrhosis found in hyperthyroidism (Rössle[13]). One patient had a slight peripheral fibrous proliferation, and four patients had fatty changes in the liver.

Of the tests running parallel with the positive T. A. R. in this series, in cirrhosis of the liver the icteric index, as discussed above, showed a parallelism with the T. A. R., but upon comparing the T. A. R. with the icteric index in the remaining groups, there was no parallelism, although it must be admitted that not all cases had the icteric index of blood serum run.

The positive Kahn and Wassermann reaction of the serum does not seem to interfere with the Takata-Ara reaction. In a total of 114 positive T. A. R. cases, 14.39 per cent gave a positive Kahn or Wassermann, and 59.89 per cent gave a negative Kahn or Wassermann. Of the negative T. A. R. cases (162), 16.19 per cent gave a positive Kahn or Wassermann and 76.88 per cent gave a negative Kahn.

This finding is in agreement with Tannenholz,[18] who states that the Takata-Ara reactions bear no relationship to the Wassermann or Meinike test. He does state, however, that the T. A. R. is more frequently positive in cases of syphilis (15 per cent) than in other cases (8.5 per cent), such as, genitourinary, dermatologic, and medical cases where a question of liver damage does not present itself.

As to the factors involved in causing the flocculation in a positive Takata and Ara reaction it has been stated by Takata,[1] by Nicole,[12] and Jezler[19] that it is the decreased stability of the serum proteins of the colloid system which makes the precipitation of the colloidal solution of mercuric oxide possible, and that this is due to the increase of globulin with an inversion of the albumin and globulin ratio. It is, according to Nicole,[12] Takata,[1] and others, albumin which exerts a protective action, thus preventing the flocculation of the colloidal solution of mercuric oxide.

In studying the albumin and globulin fractions (after Howe's method[20]), and their ratios, I found that in a total of 23 positive T. A. R. cases, seventeen cases had a ratio of lower than 2:1. If this ratio is taken as the normal, then it would appear to favor the theory of increased globulin content as a factor in causing the flocculation of the mercuric oxysol. However, when I examined 21 cases which gave a negative T. A. R. (Table II), I found that seventeen had a ratio of lower than 2:1.

The globulin in the positive T. A. R. (Table III) does not show an absolute increase, as one would expect to find, according to Jezler's work.[19] In his work on cases with positive T. A. R., the globulin percentage of the total serum proteins was higher than 55, whereas, in the negative T. A. R., the percentage of globulin of the total serum proteins was about 37.

TABLE II

PROTEIN DETERMINATION IN BLOOD SERUMS WITH NEGATIVE TAKATA-ARA REACTION

DIAGNOSIS	% OF SERUM ALBUMIN	% OF SERUM GLOBULIN	% OF GLOBULIN IN TOTAL ALB. GLOB. CONT.	ALBUMIN GLOBULIN RATIO
Lobar pneumonia	3.88	2.13	33.6	1.82
Lobar pneumonia	4.05	2.00	33.0	2.02
Lobar pneumonia	3.71	2.09	27.7	1.77
Pulmonary tuberculosis	4.36	2.11	32.6	2.06
Pulmonary tuberculosis	3.66	1.96	34.8	1.86
Pulmonary tuberculosis	4.13	2.33	36.0	1.72
T. B. pleurisy with effusion	4.39	2.36	34.8	1.86
Carcinoma of biliary tract	3.87	2.16	35.7	1.79
Carcinoma of head of pancreas	3.90	2.20	34.4	1.77
Carcinoma of stomach	3.71	2.17	31.3	1.74
Carcinoma of liver	4.11	2.19	34.7	1.87
Malignancy with metastases	4.12	2.13	34.3	1.93
Chronic glomerulonephritis	2.88	2.61	47.5	1.10
Lipoid nephrosis	2.40	2.98	55.2	0.80
Hypertensive heart disease	3.71	2.16	36.7	1.71
Hypertensive heart disease	4.00	1.93	32.5	2.07
Arteriosclerotic heart disease	4.44	2.18	32.3	2.03
Pernicious anemia	3.18	1.97	38.2	1.67
Exophthalmic goiter	3.33	2.00	37.5	1.66
Toxic hepatitis	3.33	1.94	36.8	1.71
Cirrhosis of liver	3.73	2.46	39.7	1.51

Schindel,[21] by adding various concentrations of lower fatty acids such as formic, acetic, or proprionic acids, to a dilution series of a serum, was able to produce a positive T. A. R. in a serum which otherwise gave a negative T. A. R. The addition of mineral acids, however, did not have this effect. The flocculations, according to Schindel, took place exactly as in the naturally positive T. A. R. serum. He was also able to transform the experimental as well as the naturally positive T. A. R., by the addition of sodium or potassium hydroxide, into a negative T. A. R. Nicole,[12] on the other hand, in his experiments with various concentrations of albumin and globulin derived from horse serum, was able to show that albumin is a factor in producing the various changes in color, whereas globulin is a factor in causing flocculation. When these proteins were mixed in normal proportions, the T. A. R. was negative, whereas, if the globulins were increased or the albumins decreased, a positive T. A. R. occurred.

Another interesting observation was made by Zirm,[22] who, with the addition of heparin to the blood serum, was able to prevent in a positive serum the occurrence of a flocculation, whereas a previously negative serum did not manifest any change. In his series 5 mg. of heparin per tube in one set and 20 mg. per tube in another set were used, and those tubes with the greater amount of heparin showed less flocculation than those tubes with the smaller amount of heparin.

I have carried out similar experiments, and the results are very striking, in that in some of the cases the use of as little as 1 mg. of heparin per tube

TABLE III

PROTEIN DETERMINATION IN BLOOD SERUMS WITH POSITIVE TAKATA-ARA REACTION

DIAGNOSIS	% OF SERUM ALBUMIN	% OF SERUM GLOBULIN	% OF GLOBULIN IN TOTAL ALB. GLOB. CONT.	ALBUMIN GLOBULIN RATIO
Cirrhosis of liver	4.0	1.96	32.8	2.04
Cirrhosis of liver	3.87	2.09	35.0	1.85
Cirrhosis of liver	3.91	2.10	34.9	1.86
Cirrhosis of liver	3.66	1.93	34.5	1.89
Cirrhosis of liver	3.97	2.21	35.7	1.79
Cirrhosis of liver	3.77	1.92	33.7	1.96
Cirrhosis of liver	4.26	2.08	32.8	2.04
Cirrhosis of liver	4.08	2.11	32.6	1.93
Cirrhosis of liver	4.63	2.37	33.8	1.53
Cirrhosis of liver	3.90	2.12	35.2	1.83
Cirrhosis of liver	4.11	1.95	32.1	2.10
Cirrhosis of liver	4.19	2.14	33.8	1.95
Cirrhosis of liver	4.05	1.96	32.6	2.06
Cirrhosis of liver	3.74	2.09	35.8	1.78
Syphilitic cirrhosis of liver	4.00	2.31	36.4	1.75
Acute hepatitis	4.37	2.41	35.5	1.81
Exophthalmic goiter	3.71	2.12	36.3	1.75
Exophthalmic goiter	3.84	2.31	39.1	1.66
Exophthalmic goiter	3.94	2.32	37.0	1.69
Exophthalmic goiter	3.91	1.96	34.0	1.99
Carcinoma of pylorus of stomach with metastases	3.93	2.14	35.2	1.83
Chronic malaria with severe anemia	4.50	1.97	30.6	2.28

prevents the flocculation of mercuric oxide. The following chart shows some of the results obtained.

Flocculation in tube	1	2	3	4	5	6	7	8
Cirrhosis of the liver without heparin	0	0	+4	+4	+4	+4	+3	+2
Cirrhosis of the liver with heparin, 3 mg. per tube	0	0	0	0	0	0	0	0
Cirrhosis of the liver without heparin	0	0	+3	+4	+4	+4	+3	+2
Cirrhosis of the liver with heparin, 1 mg. per tube	0	0	0	±	±	0	0	0

It is obvious from the above observations and the differences of opinion in the literature that the chemistry involved is perhaps more complicated than it seems, and it requires much further investigation to determine the actual factors concerned in the cause of flocculation in the positive T. A. R.

SUMMARY

1. Two hundred and seventy-six cases with the Takata-Ara reaction have been studied.

2. That this is of value in cases of decompensated liver conditions due to cirrhosis is without doubt. For latent liver damage the test is not entirely reliable.

3. The T. A. R. is positive in 98 per cent of the cases of cirrhosis of the liver.

4. The simplicity with which the test is carried out warrants its use in hospital and office practice as both a diagnostic and prognostic aid in cases of cirrhosis of the liver.

5. Liver impairment occurs in hyperthyroidism, as shown by the Takata-Ara reaction.

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ARTIFICIAL PNEUMOTHORAX IN THE TREATMENT OF LOBAR PNEUMONIA*

A Review of Forty Pneumothorax-Treated Patients and One Hundred Patients Treated by Other Methods

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IN A RECENTLY published article one[1] of us reported the results obtained in the treatment of eleven cases of lobar pneumonia by artificial pneumothorax. It was emphasized at that time that, although the procedure gave promise of being a worthy addition to the armamentarium in the treatment of lobar pneumonia, the number of cases treated was too small to enable us definitely to evaluate it. We have now had the opportunity to observe carefully forty patients treated by artificial pneumothorax.

Inasmuch as any study of this nature is of little value without the benefit of a control group of cases taken at the same time of the year, and under as nearly similar conditions as possible, 100 cases of lobar pneumonia treated by methods other than pneumothorax are also presented.

THE THEORY OF THE ACTION OF ARTIFICIAL PNEUMOTHORAX IN PNEUMONIA

Lilienthal[2] has suggested that since care is taken not to produce a positive intrathoracic pressure, partial collapse rather than compression occurs when air is introduced. Partial collapse is probably the most accurate way of describing the state of a pneumonic lung treated by artificial pneumothorax. In any case, a lung partially or completely surrounded by a layer of air is splinted to a lesser or a greater degree. This has been shown roentgenographically and at postmortem. The acutely inflammatory tissues of the diseased lung acquire at least a relative degree of rest because complete expansion is prevented. The parietal and visceral pleurae are separated and the pain of attendant pleuritis is abolished almost without exception. These facts we know because they have been proved clinically again and again.

The ability of air to produce even a partial degree of collapse in a pneumonic lung that is in the stage of hepatization has frequently been questioned. Clinical, roentgenographic, and postmortem findings lead us to the belief that partial collapse of such a lung can occur. The term "hepatization" brings to mind the tense and sharply defined lung as it lies on the autopsy table. We question whether this mirrors the true condition of such a lung during life. In death

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the cellular and fluid exudates rich in protein content are congealed. Such a lung would indeed render collapse impossible. That body fluids high in protein content frequently gel when removed from the body is common knowledge. Similarly the hepatized lung may be regarded as a sac containing a large amount of fluid rich in protein and a relatively small amount of air. That such an organ can be partially collapsed is possible. That it can be collapsed sufficiently to prevent further pleuritic pain, we know.

How does pneumothorax bring about crisis in lobar pneumonia? That it does so in a certain number of cases we feel relatively sure. Here is ample field for theoretical speculation and laboratory investigation. It has been shown that artificial pneumothorax causes a decrease in the amount of blood circulating in the lung so treated. Lymph stasis with diminution in the absorption of toxins has also been shown to occur after pneumothorax. Whether or not crisis is the product of the sum of these factors acting in conjunction with the natural immunologic resources of the organism can only be surmised.

More or less subjective improvement promptly followed artificial pneumothorax in the majority of cases of lobar pneumonia so treated, irrespective of the ultimate outcome. The reaction in some is clinically similar to that seen after the intravenous injection of a foreign protein, except for the absence of the initial chill. The critical fall in temperature and the objective and subjective improvement in the patient lead one to speculate on the cause. One of us (W. E. R.) has been inclined to regard it as a protein reaction induced by the air, which very probably forces altered proteins into the blood stream from the involved lung. Differential blood counts, however, do not entirely support this view, for the well-known phase reactions following intravenous protein injections, are not so pronounced a feature after pneumothorax.

SELECTION OF CASES FOR ARTIFICIAL PNEUMOTHORAX

All the patients treated in this series were believed to have unilateral lobar pneumonia involving one or more lobes at the time pneumothorax was induced. As soon as the diagnosis was established by physical signs or confirmed by roentgenographs, air was introduced. In an effort to determine the true value of the treatment, all patients admitted to our services that fulfilled the requirement of unilateral lobar pneumonia were given air. Some of the patients appeared to be doomed at the time treatment was begun. Occasionally this type of patient surprised us by recovering, but usually the treatment was of no avail in preventing death. Seven patients were women, thirty-three were men.

Patients were given air regardless of the day of disease. This varied from the first to the fourteenth day, although the first pneumothorax in the majority of cases was given on the third, fourth, or fifth day. Many of the most brilliant results occurred in this group. It seems to us that those who use artificial pneumothorax only on or before the third day, as has been advocated, limit its application needlessly and deny the patient therapeutic aid at a time when it is best utilized and when the response of the patient is most gratifying.

The amount of air given was usually between 400 c.c., and 600 c.c. and in most cases was repeated in twenty-four hours. This amount was used because

it is sufficient to accomplish the desired result without causing discomfort to the patient or a mediastinal shift. At no time was a positive intrapleural pressure produced, the needle being always withdrawn before this stage was reached. Occasionally it was impossible to introduce more than 200 c.c. of air because of pleuritic adhesions. In only one case did adhesions altogether prevent the establishment of pneumothorax.

SELECTION OF CASES FOR CONTROL GROUP

The first one hundred patients admitted to the wards with lobar pneumonias after Dec. 1, 1933, and not treated by pneumothorax, comprise the control group. Of these, eighty-four were men, sixteen were women. Since there are ten medical services at the Philadelphia General Hospital, and artificial pneumothorax was used on only one or two, controls were abundant. Specific serums could only be given when the patient or his friends could afford to buy it, so that it was used in only two cases. One of these had a nonspecific immunotransfusion in addition. Another patient received nonspecific protein therapy in the form of 50,000,000 killed typhoid organism intravenously. The remainder of the patients were treated by the usual supportive measures and those designed to give symptomatic relief.

RESULTS OF TREATMENT

The results of treatment are presented in compact form in Table I. A few facts are worthy of special mention.

TABLE I

SUMMARY OF DATA

AGE, YEAR RANGE	SEX		COLOR		MODE OF FALL OF TEMPERATURE		ORGANISM											DEFERVESCENCE, MEAN, DAYS	ARTIFICIAL PNEUMOTHORAX			DIED	RECOVERED
							SPUTUM					BLOOD											
							PNEUMOCOCCUS					PNEUMOCOCCUS											
	M	F	W	B	LYSIS	CRISIS	TYPE 1	TYPE 2	TYPE 3	GROUP 4	B. FRIEDLANDER	TYPE 1	TYPE 2	TYPE 3	GROUP 4	B. FRIEDLANDER	UNKNOWN	NO GROWTH		DAY AFTER ONSET OF	AIR INJECTED, RANGE C.C.		
Cases 1 to 40 inclusive, treated by artificial pneumothorax																							
13-69	33	7	18	22	16	18	3	4	0	13	1	2	1	0	8	0	0	11	3.42	4.2	50-600	14	26
Cases 41 to 140 inclusive, treated by other methods																							
12-75	84	16	63	37	38	9	1	5	0	12	0	4	11	2	5	1	1	15	8.57			51	49

Relief of Pain.—That artificial pneumothorax relieves the pain so frequently associated with respiration in lobar pneumonia all observers agree. Relief is experienced almost immediately after the needle is withdrawn and is promptly noticed by the patient. The need for narcotics is in consequence greatly diminished. Relief of pain is one of the most spectacular results of the use of artificial pneumothorax in the treatment of lobar pneumonia. One of us (V. L.

T.), who treated twenty-five of the forty cases in this series, has noted that patients frequently requested refills of air after rapid absorption had allowed a partial return of pleuritic pain.

Relief of Cyanosis, Dyspnea, and Toxicity.—Cyanosis is less frequently encountered than pain in early pneumonias. It is our experience that when present it is relieved by pneumothorax. Dyspnea is frequently associated with pain. When pain is relieved by the introduction of air, dyspnea usually disappears. "Toxicity" is a rather abstract term, and has to be judged by the appearance and the mental state of the patient. In one patient jaundice was present on admission but disappeared within twenty-four hours. When a toxic psychosis is well established, pneumothorax will not abort it, but we have seen patients apparently on the threshold of delirium benefit remarkably and take a renewed interest in themselves and their surroundings. It is certain that, in general, patients look less sick after pneumothorax has been induced, but when a toxic psychosis does occur, it makes the prognosis decidedly more grave.

MORTALITY

In the control group of one hundred cases, there were fifty-one deaths, a mortality of 51 per cent. This is an appallingly high death rate, and yet it is substantially in accord with figures which are reported from other large general hospitals. Here is evidence enough that there is need now, as there has been for the past two hundred years, for an effective agent in the treatment of lobar pneumonia, an agent that must be inexpensive, yet potent without regard to the type of organism causing the infection.

In the pneumothorax-treated group there were fourteen deaths, a mortality of 35 per cent. Three of these died within eight hours after admission to the hospital, and were almost moribund when treated. That we have been able to reduce mortality by 16 per cent in the worse types of pneumonia suggests the hope that those who treat more favorable cases can do even better.

One of the deaths occurred in a patient with a toxic psychosis whose temperature fell from 104° to 96° twelve hours after the second injection of air. The psychosis persisted despite the febrile drop and the patient inadvertently got out of bed that day. He died a few hours after he was put back to bed.

Another death occurred in a patient who had been afebrile for five days. During all this time he had a low muttering delirium from which it was impossible to arouse him, and death was apparently due to myocardial exhaustion. There was no postmortem examination.

Two cases showed acute vegetative endocarditis at autopsy.

PERIOD OF DEFERVESCENCE

Table I shows the mean duration of fever during the hospitalization of patients who recovered. After the first pneumothorax the average duration of fever was three and a half days. In the control group the average duration of fever was seven days. It appears, then, that pneumothorax shortens the febrile course. This in turn means an abbreviated convalescence and conservation of

the energy reserves of the patient. In addition, the economic benefit which the patient and those who pay for his hospitalization derive from his shortened stay is notable.

PRODUCTION OF CRISIS

In eighteen of the forty patients treated by induced pneumothorax, the temperature fell by crisis, using the term to denote a fall in temperature to normal within twenty-four hours after the first injection of air. The temperature does not then remain normal in all cases, and this is the main reason for repeating the treatment within twenty-four hours. Two injections usually suffice, but there should be no hesitation in giving others if they seem indicated. The fact that crisis is induced does not of necessity imply that the patient will recover. Six of the eighteen patients died despite that fact, the temperature usually rising again after a preliminary drop, and remaining elevated despite the addition of more air.

In sixteen cases temperature fall by lysis was observed. It was impossible to predict whether temperature would fall by crisis or by lysis.

In six cases pneumothorax produced no temperature change and in one of these only 150 c.c. of air could be introduced, because of the presence of adhesions.

Among the one hundred patients treated by methods other than pneumothorax, spontaneous crisis occurred only seven times. These patients all recovered.

COMPLICATIONS

In the pneumothorax-treated group:		In the control group:	
Delayed resolution	1	Thrombophlebitis	1 (recovered)
Pericarditis	2 (both recovered)	Suppurative otitis media	1 (died)
Acute vegetative endocarditis	1 (died)	Jaundice	1 (died)
Empyema	1 (recovered)	Pneumococcic meningitis	1 (died)
Pneumococcic meningitis and acute vegetative endocarditis	1 (died)	Pleural effusion	3
		Acute arthritis	1
Jaundice	1 (recovered)	Empyema	3 (all recovered)
		Lung abscess	2 (1 died, 1 recovered)
		Delayed resolution	3
		Acute vegetative endocarditis	2 (died)

There is apparently little difference in the occurrence of complications in the two series (17 per cent in pneumothorax-treated cases, 18 per cent in those treated by other methods).

RESULTS OF BLOOD CULTURE

In patients with an established pneumococcemia artificial pneumothorax was of little benefit. Five of the 40 patients treated returned positive blood cultures, and of these only one recovered.

In the control group of 100 cases there were 27 cases of bacteremia and of these patients 6 recovered and 21 died.

The results in this group are rather striking, and may or may not be significant. According to our figures, the number of cases of bacteremia occurring in the cases treated by artificial pneumothorax was less than half (12 per cent) that of the number that appeared in the control group (27 per cent).

SUMMARY

A series of 40 patients with lobar pneumonia treated by artificial pneumothorax is presented, with a mortality of 35 per cent. A control group of 100 patients with lobar pneumonia treated by other methods is also presented, with a mortality of 51 per cent. In addition to a decreased mortality, the chief benefits of artificial pneumothorax are relief of pain, dyspnea, cyanosis, and "toxicity." It also shortens the febrile period sometimes by crisis, sometimes by lysis, and so lessens the number of hospital days per patient. Artificial pneumothorax is of little aid in the treatment of well-established pneumococcemias; it will not abort toxic psychoses, and it apparently has little influence on the prevention of the more severe complications of lobar pneumonia.

CONCLUSION

One must conclude from the results of this study that artificial pneumothorax offers greater hope for recovery, greater comfort during illness, and a shorter period of convalescence than the ordinary nonspecific measures which strive for symptomatic relief in patients suffering from unilateral lobar pneumonia.

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PHOSPHORUS METABOLISM

V. Relation Between Urinary Phosphate and Blood Phospholipids During Absorption of Fats

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IT HAS been shown by a number of investigators that the phospholipids of the blood of animals increase on a high-fat meal. In dogs Bloor[1] found an increase in whole blood of as high as 70 per cent and never less than 30 per cent, when 50 to 100 c.c. of olive oil alone was fed. Recently[2] he found that in single overfeedings of dogs with fat or carbohydrate the increase in plasma phospholipids averaged 28 per cent. There was no increase with protein. So far as the phospholipids of the human blood are concerned it is known that the content, under similar conditions of food, environment, etc., is about the same as in dogs and many other animals.

Assuming that the whole blood increases about 33 per cent in phospholipid within about four to six hours after a meal (from a content of 0.3 up to 0.4 per cent), in other words an increase of 0.1 per cent, we can calculate that the blood of an average person would then contain 4.5 gm. more phospholipid than previously (0.001 by 4,500 c.c.). Since the phospholipids contain about 4 per cent of phosphorus, the increase represents 180 mg. of that element. This is not an inconsiderable quantity, and if it is derived directly from the same phosphorus compound in the body as the urinary phosphate, we might expect the latter to be decreased. The source for urinary phosphates is in all probability the inorganic phosphate of the blood,[3] although evidence has also been presented especially by Eicholtz, Robison and Brull[4] which makes it somewhat inconclusive. Whatever the facts are in this regard, if the blood phospholipid increase represents newly-formed phospholipids, we would expect that the P from both they and the urinary phosphates would be derived from the same immediate and potential source. Thus, an increase in blood phospholipids would result in a decrease in urinary phosphates.

The purpose of this investigation was to determine if the blood phospholipid increase in reality does decrease the urinary phosphate.

EXPERIMENTAL

Four normal men twenty-one to twenty-five years of age were selected as subjects. Their urines were carefully collected in one-hour periods for eight consecutive hours, beginning at 7 A.M., with the usual breakfast of each subject soon after 8 A.M. The usual amount of water was allowed but no other food than the breakfast. The volumes and the phosphorus contents of the urines were determined. The results are shown in Table I. The table also shows the results

*From the Department of Biological Chemistry, University of Buffalo Medical School. Received for publication, October 5, 1934.

TABLE I*

INORGANIC PHOSPHORUS IN URINE OF MEN, ON USUAL AND ON HIGH-FAT BREAKFASTS (MG. P PER HOUR)

TIME	SUBJECT M		SUBJECT C		SUBJECT A		SUBJECT H		TOTAL	
	USUAL BREAKFAST	HIGH-FAT BREAKFAST	USUAL BREAKFAST	HIGH-FAT BREAKFAST	USUAL BREAKFAST	HIGH-FAT BREAKFAST	USUAL BREAKFAST	HIGH-FAT BREAKFAST	USUAL BREAKFAST	HIGH-FAT BREAKFAST
7-8 A.M. breakfast	46.2	77.2	51.8	98.0	39.1	27.3	44.4	40.6	181.5	243.1
8-9	19.3	20.1	20.8	29.1	28.4	32.2	48.6	36.1	117.0	117.5
9-10	6.2	7.4	20.8	51.6	19.9	30.5	24.1	32.2	71.0	121.7
10-11	21.1	5.5	14.6	39.8	28.0	28.9	22.5	28.9	86.2	103.1
11-12	30.8	15.8	18.7	41.1	25.8	51.6	27.6	23.7	102.9	132.2
12-1 P.M.	36.5	36.2	28.1	11.4	37.3	32.4	26.2	24.5	128.2	104.5
1-2	40.3	8.7	24.5	19.5	45.0	38.0	19.8	44.5	129.7	110.7
2-3	31.9	37.1	21.7	41.5	44.0	38.6	26.7	45.9	124.4	163.1
Total	232.4	208.0	200.9	332.0	267.6	279.5	240.0	276.4	940.9	1095.9

*Body weight: Subject M, 76.4 kg.; C, 64.5 kg.; A, 71.8 kg.; and H, 88.2 kg.

of a similar run with the same subjects a week later, except that during the breakfast 75 gm. of corn oil (Mazola) was ingested by each. This amount of oil is about the maximum that can be taken by the average person without causing considerable nausea or laxation.

RESULTS AND DISCUSSION

The tabulated results show that the formation of urinary phosphate is not influenced or diverted to form phospholipid in the body when a high-fat meal is taken; there is even somewhat more phosphate excreted under the fat meal. It can be observed on closer inspection of the table that there are decreases in phosphorus outputs during some parts of the eight-hour periods under the fat meals, but the decreases are irregular and cannot be ascribed to phospholipid formation from absorbed fat. Therefore it cannot be concluded that the phosphorus of blood phospholipid and urinary phosphate are drawn from the same immediate source.

There is no correlation between excretion and urine volume. This is already well known and the figures for the urine volumes are therefore not included.

Most investigators have accepted the phospholipid rise in the blood during fat absorption as a neogenesis for purposes of transportation and intermediary metabolism of the fatty acids. This has been the starting point for extensive consideration of the phospholipids in this regard. Recently Sinclair,[5] on the basis of his experiments, has suggested the hypothesis that the absorbed fatty acids are transformed into phospholipid within the intestinal mucosa as an essential step in the resynthesis of neutral fat. His data indicate that there is, however, no increase in phospholipid content in the intestinal mucosa when fats are absorbed. It seems possible that neogenesis in the wall during fat absorption results in phospholipid flow into the blood and that this accounts for the nonrise in the mucosa. The phospholipids could thus function both in fat absorption and fat transportation. (Other possible functions of phospholipids do not appear to be connected with this research.)

The results of this research indicate the probability that the increase in blood phospholipids represents such lipids mobilized from some other part of the body, whether it be from the intestinal wall, the liver, or the spleen, for example, and that this increase is not closely connected with the excretion of phosphates in the urine.

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COMPLEMENT FIXATION REACTIONS IN CARCINOMA*

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A CONSIDERABLE number of investigators have studied the alcohol soluble heterophile antigen extractable from carcinomatous tissue and have concluded that there exists a cancer specific antigen. In large part, these conclusions have been reached as a result of experiments in which the antigen, more or less purified by solution or precipitation with various organic solvents, has been injected into rabbits and a specific antibody had been shown to develop. In addition, efforts have been made to demonstrate by means of the complement fixation test the presence of antibodies in the blood of cancerous patients which react specifically with the heterophile carcinoma antigen.

Hirszfeld and Halber[1] announced in 1930 that in 10 or 20 per cent of rabbits which were injected with alcoholic extracts of cancer tissues, antibodies were obtained which reacted with 60 per cent of all tumors tested while these antibodies did not react with normal tissues. They also tested 300 cancer and 1,700 normal serums with cholesterinized alcoholic extracts of cancer tissue and found 50 to 60 per cent of these serums positive in cases of cancer of the inner organs but only rarely in cases of cancer of the external tissues. If the serums were Wassermann positive, they were also positive with the cancer antigen.

Witebsky and Pöplau[2] showed that cancer tissue might be boiled for thirty minutes without losing the ability to produce specific antibodies when injected into rabbits. They found that subsequent to the injection of cancer material in animals, the development of cancer specific serum alterations depend mainly on the particular cancer material used but also on the individual rabbit.

Cohn and Collier[3] found no antibodies resulting from the injection of rabbits with the plain alcoholic extract but when they mixed the extract with pig serum before injecting it specific antibodies were temporarily produced. Sievers[4] found reactions occurring with both immune and normal rabbit serums when using cancer extracts. He believes that the complement fixation results from a physical-chemical change. Recently, Saphir and Hirschberg,[5] using cholesterinized alcoholic extracts, found positive fixations in 77.7 per cent of 27 cases of cancer. Only one case out of 82 normal controls was positive. All of the serums which they tested were Wassermann negative. These workers also think that the reaction is of a physical-chemical nature due to the action of the antigen on the serum globulin.

The older tests were negative for specificity because the crude alcoholic extract which was used as antigen contained mixtures of various substances. These substances react with a variety of antibodies and it is only by their removal that specific reactions may hope to be obtained.

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A distinct advance was made by Lehmann-Facius.[6] He realized the necessity of purifying the antigen and so fractionated the alcoholic extract by use of certain organic solvents. That fraction which was soluble in ether, benzene and alcohol but insoluble in acetone he found to contain the cancer antigen. This fraction he thought to be a phosphatid. By means of this fractionation he got rid of certain fatty acids, fats and cholesterol esters and greatly increased the reactivity of the antigen. Like previous workers he found that syphilitic serums likewise gave positive reactions with the extract.

Morelli,[7] using a limited number of extracts, reported that she could not separate the various fractions by this method of Lehmann-Facius.

In the present work two points have been studied: first, does the heterophile syphilitic antigen give specific positive reactions with the bloods of cancerous patients? Second, is it possible to separate the syphilitic antigen present in the cancer tissue extract from the specific cancer antigen?

The method of preparation of the antigen used in these experiments was essentially the same as that recommended by Lehmann-Facius. A fresh specimen of cancer tissue was obtained from the operating room or autopsy room and all normal tissue and fat removed with the aid of scissors. The tissue was then ground in a food grinder and placed in a flask containing glass beads together with five times the volume of 95 per cent alcohol. The tissue was then extracted at 37° C. for eight days, shaking the flask several times a day.

At the end of this time the extract was filtered through a double filter paper and the filtrate constituted the alcoholic extract. For purposes of fractionation 40 c.c. were placed in a large test tube and evaporated to dryness in a water-bath. The residue was dissolved in 40 c.c. of benzene, the solution filtered and the filtrate again evaporated. The residue was dissolved in acetone and the insoluble fraction collected by centrifuging the mixture. This insoluble portion was then dissolved in petrol ether, filtered and again evaporated. The residue was dissolved in 95 per cent alcohol, the solution filtered and the final volume adjusted to one-half of the original. This fraction corresponds to the phosphatid fraction of Lehmann-Facius.

In the later antigens which were prepared, ether and chloroform were used in the extraction in addition to the other solvents as a further purification of the antigen was apparently obtained when these solvents were employed. The active material was soluble in these reagents. These solvents were used after the petrol ether.

Ethylene chloride was also employed as a solvent. The value of this reagent was discovered while conducting a series of experiments with solvents, having higher boiling points, on the destruction of the antigen by heat. It apparently gives quite promising results. The acetone insoluble fraction was dissolved in one-half the volume of ethylene chloride, the solution filtered and the filtrate evaporated as before. The residue was dissolved in one-fourth the original volume of 95 per cent alcohol and the resulting antigen was quite active even without cholesterinization.

By extracting each antigen several times either with each reagent or with certain reagents a further purification might be accomplished. This procedure was employed in the more recent extractions and it was found to be of value.

When the antigen was kept for periods longer than a month it became increasingly anticomplementary and in spite of dilution it was no longer usable.

Cholesterinization of some of the antigens was carried out and was performed by using the technic of Hirszfeld and Halber.[8] To 0.35 c.c. of the extract was added 0.15 c.c. of a 1 per cent alcoholic solution of cholesterin. Three cubic centimeters of saline were then quickly added and the material mixed with shaking. In this form the antigen was very unstable, and it had to be prepared just before it was used.

A Wassermann antigen was also prepared according to the method recommended by Kolmer and the cancer serums were all tested for their reaction with the syphilitic antigen.

The patient's blood was inactivated at 56° C. for twenty minutes before using it. This inactivation was carried out very carefully as heating a positive serum at 58° for fifteen minutes seemed to destroy the antibodies reacting with the cancer antigen. In all the experiments increasing dilutions of the serum from cancer patients were used in testing the cancer antigens. A 2 per cent suspension of sheep cells was used in the indicator system. Two units of amboceptor contained in 0.5 c.c. were used in the set-up.

The antigen was titrated for its hemolytic, anticomplementary and antigenic power. It was used in a dilution which was ten times the titrated strength, thus ten units were used; this value was always removed by at least ten or more units from the hemolytic and anticomplementary titers or the antigen was considered unfit for use.

For the test proper nine tubes were set up for each serum to be examined. The first four contained serum dilutions of 1-5, 1-10, 1-20, and 1-40, respectively. The fifth tube contained a 1-5 dilution of the serum but saline was added in place of the antigen. The sixth tube contained four-plus syphilitic serum diluted 1 in 5 and the seventh the same serum but saline in place of the antigen. The eighth and ninth tubes contained normal serum diluted 1 in 5, with saline replacing the antigen in the ninth tube. The complement was titrated each day just before using it. Readings of the final results were noted as four-plus, three-plus, etc., depending on the degree of inhibition of hemolysis.

Twenty-one antigens were made according to the Lehmann-Facius technic. Not all of them were suitable for use, however, as some did not exhibit sufficient difference between the fixing unit and the anticomplementary unit to meet the requirements specified above. As a rule, the hard, firm tissue made the best antigen; the softer necrotic tissue was less serviceable. Cancer of the inner organs provided better antigens than did growths from the outer tissues. This is in agreement with the results obtained by Marzynsky and Silberstrom[9] and opposite from those of Hirszfeld and Halber.

Of seven tumors of the breast only two furnished satisfactory antigens; of six tumors of the skin only one furnished a satisfactory antigen; of three tumors of the gastrointestinal tract (stomach, colon and rectum) all gave usable ex-

TABLE I

COMPLEMENT FIXATION BY SERUM FROM CANCER CASES USING AS ANTIGEN "PHOSPHATID" ANTIGEN FROM CANCERS

LOCATION OF TUMOR	TOTAL	TOTAL NUMBER POSITIVE	NUMBER POSITIVE AND EARLY CASE	TOTAL NUMBER NEGATIVE	NUMBER NEGATIVE AND EARLY CASE
Breast	12	12	–	–	–
Breast	1	–	–	1	–
Metastasis from body of uterus	4	4	–	–	–
Cervix uterus	5	5	–	–	–
Rectum	6	5	–	1	–
Stomach	1	–	–	1	1
Skin (various locations)	8	2	1	6	5
Buccal cavity	2	2	2	–	–
Tongue	2	–	–	2	2
Larynx	1	1	–	–	–
Esophagus	2	1	1	1	1
Unknown	7	4	–	3	1
Totals	51	36	4	15	10

tracts; three metastatic tumors (lung, liver and pelvis) also gave good antigens; and one tumor of the testicle gave a good antigen.

The antigenic titer of the acetone insoluble fractions of the antigen varied between 1.150 and 1.1500 for cancer serum and between 1.50 and 1.100 for syphilitic serum. The selected cholesterinized antigens fixed the complement in titers of 1.400 to 1.4000 in the presence of cancer serum and between 1.300 and 1.2000 with syphilitic serum. The ethylene chloride fraction gave a titer of 1.4000 to 1.5000 with the cancer serum and less than 1.1000 with syphilitic serum. It is apparent that quantitatively there does seem to be a certain limited degree of specificity of the cancer antigen. This was, however, not evident in all the cancer antigens. It also appears that the ethylene chloride fraction shows this specificity more markedly than the other less highly purified antigens.

The serums from fifty-one cases of carcinoma were tested with Lehmann-Facius' phosphated antigen, either with or without addition of cholesterin and positive reactions obtained in about 70 per cent (Table I). All of these serums were Wassermann negative. One hundred and seven syphilitic serums which gave a 4+ reaction with the Kolmer antigen were examined with the same antigens and all but two of them reacted positively with the acetone insoluble fractions prepared according to Lehmann-Facius. With ninety-two normal control serums one positive reaction was obtained. Ten serums from tuberculous patients gave negative reactions as did two from cases of adenoma of the breast.

These results compare quite favorably with those of Simon and Thomas[10] and Hirszfeld and Halber. As has been stated, it is generally accepted that the alcoholic extracts from cancer tissue will react with syphilitic serums and even upon fractionation of the antigen not all of the syphilitic antigen is removed. Our results also bear out the claims of Lehmann-Facius that a more active, purified form of the antigen may be obtained by the method of fractionation used.

The patient's serum gave stronger reactions when the involvement by the growth was more extensive. There was quite a close correlation between the extent to which the cancer had advanced and the reaction obtained. Practically

all of those serums giving negative reactions were from patients upon whom the diagnosis of cancer in the early stages had been made.

The use of ethylene chloride in the fractionation allowed an earlier detection of cancer by this reaction than did the plain phosphatid fraction. Serums that were negative with the latter antigen gave two- and three-plus reactions with the ethylene chloride antigen. Only three satisfactory antigens were prepared by this method and twenty-eight serums tested so that the results are not as valuable as if a greater number had been used. All twenty-eight serums were, however, positive. Several of these serums were from cases of early carcinoma and some were from cases which had given a negative reaction with the Lehmann-Facius phosphatid antigen.

Twenty-seven syphilitic serums were tested with these three ethylene chloride antigens and sixteen were positive while eleven were negative. This apparently indicates a purification of the cancer antigen and removal of some of the Wassermann antigen.

On the other hand, out of eleven normal serums tested with this purified antigen, four gave positive reactions. This would seem to indicate that the purification of the antigen had made it too reactive.

The fact that syphilitic serums gave reactions with extracts from cancer tissue was recognized quite early by the men in this field. Hirszfeld and Halber, Witebsky and Pöplau, Marzynsky and Silberstrom, Lehmann-Facius and others all finding fixation with syphilitic serums. This may be explained either on the basis that the cancer antigen contains the syphilitic antigen also or that the syphilitic serum contains the cancer antibodies. Lehmann-Facius believes the former to be true and this is borne out in the present work.

On the other hand, Hirszfeld and Halber[11] think that it is the antigen found in the blood Group A or AB individuals that is active. In order to check this concept antigens were prepared by alcohol extraction of erythrocytes of several Group A individuals and also one Group B individual. None of the antigens so prepared gave positive complement fixation with either serum from syphilitic or cancerous individuals. In the present work all but six of the bloods from cancer patients were typed, but as far as could be determined the grouping did not influence the results.

In typing the bloods of the cancer patients in regard to the blood groups it was noticed that there was an unusually high number of individuals falling in Group A. This fact does not seem to be related to any known factor. Of the 45 persons typed who were suffering from a cancer, 55 per cent were in this group.

A further indication that both syphilitic and cancer antigens are present in the cancer extract is the fact that the cancer antigen is destroyed by certain alkaline reagents while the syphilitic antigen remains intact. This was accomplished by dissolving the acetone insoluble residue of the cancer extract in pyridine, filtering, dissolving the residue obtained after evaporating the pyridine in amyl alcohol, filtering of the insoluble portion and evaporating off the amyl alcohol. When this residue was dissolved in 95 per cent alcohol and tested for its antigenic power with cancer positive serum, the fixing ability had entirely

disappeared. The same is true when using ethylene diamine, and yet fixations were obtained with these same fractions with syphilitic serums. When ethylene glycoll is used, both the antigens are destroyed.

The destruction of the cancer antigen was shown not to be due merely to the heat necessary to evaporate the solvent. By using distillation in the vacuum chamber the liquids boiled under 70° C. and still the cancer antigen was destroyed. By heating the acetone insoluble fraction in a cotton seed oil bath, it was found to be stable at 125° C. for half an hour and gave fixation with cancer and syphilitic serum. From these results it would seem that both the cancer antigen and the syphilitic antigen are present in the cancer extract and that they have characteristically distinct properties.

If this assumption is made, the problem still remains to separate the two substances and the most promising method for accomplishing this seems to be fractionation and repeated extraction with various organic solvents. There is the danger, of course, that this may be carried too far and one may obtain a nonspecifically sensitive antigen. The ethylene chloride fraction gave several positive reactions with normal serums, and this is a factor which must be taken into consideration. With the phosphatid fraction only one normal control serum gave a positive test.

It seems evident from these studies that the antigens which are active in syphilitic complement fixation reactions and in cancer fixation reactions are probably not identical, and it should be possible by further study to separate these two antigens. It does not necessarily follow that the complement fixation reaction with the cancer antigen will prove to be of clinical value but certainly further study is suggested.

CONCLUSIONS

By fractionating according to Lehmann-Facius the alcoholic extract of cancer tissue, positive complement fixation reactions were obtained in 70 per cent of 51 cases of carcinoma examined. Of 28 carcinoma serums tested with the ethylene chloride antigen, 100 per cent were positive. With the cholesterinized phosphatid fraction of the alcoholic extract all but 2 of 107 four-plus syphilitic serums reacted positively. With the ethylene chloride fraction, out of 27 four-plus syphilitic serums tested, 11 were negative. Out of 92 normal serums one gave positive reactions with the phosphatid fraction while out of 11 such serums 4 were positive with the ethylene chloride fraction.

The antigen concerned in fixation with cancer serums is not destroyed by heating to 125° C. but is destroyed by certain alkaline reagents. The cancer antigen apparently is different from the syphilitic antigen, and it appears probable that these two antigens may be separated.

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THE SERUM CALCIUM IN ARTHRITIS*

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WHILE it is generally accepted at the present time that arthritis is a constitutional disease, it is equally true that the most striking clinical manifestations are those in the bones and joints. The ordinary clinical classification into atrophic and hypertrophic forms bears witness to the variety of the osseous changes. In the rheumatoid or atrophic form of arthritis there is a generalized decalcification and rarefaction of the bones involved. This is most marked at the epiphysis but usually involves the cortex of the shaft to a lesser degree. In osteoarthritis or hypertrophic arthritis the process of decalcification is not so marked, but there is a simultaneous deposition of new bone which produces calcified excrescences at the chondro-osseous junctions and eburnation at the denuded areas of epiphysis. It is claimed that the fundamental and initial lesion in osteoarthritis is one of erosion of the articular cartilage and that the osseous changes are secondary and perhaps compensatory in character. There is no question, however, that the metabolism of the bone is altered in both forms of arthritis.

The study of changes in the metabolism of bone is difficult. The mineral balance of the body is not easy to determine, and there have been only a few cases of arthritis in which the calcium balance has been determined satisfactorily. The results in these latter have been, for the most part, inconclusive. This perhaps is to be expected, for arthritis is a chronic disease and a disturbance in the calcium metabolism, too small to be demonstrated conclusively in a balance experiment of a few days' duration, would produce cumulative effects of a marked and extensive character in the course of months or years.

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Further attempts have been made to demonstrate changes in calcium metabolism in arthritis by a study of the calcium content of the blood serum. Previously reported data on this subject have been contradictory and inconclusive. Weil and Guillaumin,[1] Watchorn,[2] Horowitz,[3] and Mark[4] all report the serum calcium to be increased in arthritis and especially in arthritis deformans. Ssamarin,[5] Copp,[6] Funsten,[7] and Morelle[8] report that the serum calcium is normal in many cases, but that it may be elevated in a proportion of the cases especially in osteoarthritis. This proportion varies from an occasional case according to Morelle who not only reported a series of twenty cases but collected many others from the literature, to two-thirds of the cases reported by Ssamarin and Copp. Other investigations as Cajori and Crouter,[9] Jung and Hakki,[10] Bastos and Mazo,[11] Gold,[12] Nachlas,[13] Bauer,[14] or Battistini and Robecchi[15] report that the serum calcium is normal in the various types of arthritis. The determination of the behavior of serum calcium in arthritis is important, for the rôle of the parathyroid glands in regulating calcium metabolism has been fully established and an elevation of the serum calcium is one of the diagnostic points in the syndrome of hyperparathyroidism produced by tumors of the parathyroid glands. Even in the absence of parathyroid tumor various surgeons as Oppel[16] and Ballin[17] have argued that the finding of an elevated serum calcium in a case of arthritis is evidence of hyperactivity of the parathyroid glands and have surgically removed one or more of the latter. This contention has been a source of a great deal of controversy. That there is any relationship between overactivity of the parathyroid glands and arthritis has been denied even more emphatically than it has been affirmed. It should not be affirmed, or parathyroidectomy undertaken, on the basis of a single determination of the serum calcium.

Our own experience has convinced us that the determination of the serum calcium is liable to technical error. If the technic is carefully standardized and rigidly carried out extremely uniform results are obtainable. Unless this is done, however, very different results may be obtained, in different laboratories or even by two technicians in the same laboratory, without obvious analytical error in either case. Because of this factor the demonstration of an elevation of the serum calcium in any disease is much more significant if compared with a series of control determinations made in the same laboratory. Furthermore, as emphasized by Bauer the serum calcium is continuously elevated in cases of parathyroid tumor. A single elevated determination in a series done on one patient, is more likely to be due to technical error than to the transitory parathyroid hyperactivity as reported by Funsten.[7]

Many of the investigators as Cajori and Crouter, Nachlas, and Bauer, who have reported normal values for the serum calcium in arthritis have not reported their data in detail or else the number of cases studied was too small to make the data authoritative. We have, therefore, attempted to answer these questions by a study of the calcium content of the serum in a sufficiently large group of cases of arthritis to permit of a statistical analysis of the results.

MATERIAL AND METHODS

The subjects for this study were patients in the Arthritis Clinic of the New York Post-Graduate Hospital. A series of fifty cases of rheumatoid arthritis and a like number of cases of osteoarthritis were taken in sequence. Blood specimens were obtained by venipuncture two or three hours after breakfast. The serum calcium was determined by the Clark-Collip Modification of the Kramer-Tisdall Method. The cases therefore all came from one clinic and the determinations were done in one laboratory.

CONTROLS

The usually accepted normal range of variation for the serum is between 9.0 and 11.0 mg. per 100 c.c.[18, 19] Various authors have reported small groups but the largest control group is that of 852 cases reported by Greene and Boothby[20] from the Mayo Clinic. In the present series we have studied a group of fifty hospital cases in which there was no obvious metabolic or other disturbance which might affect calcium metabolism and a second group of 128 ambulatory cases referred from the private practice of various staff members. These cases were not especially selected and so may be expected to show a greater degree of variability than our first group. The formulas used in the statistical analyses of our results are to be found in the standard textbooks on statistics.[21, 22]

RESULTS

The serum calcium of fifty cases of rheumatoid arthritis is given in Table I. The mean and standard deviation of this group was 10.218 ± 0.699 mg. per 100

TABLE I

THE SERUM CALCIUM IN RHEUMATOID ARTHRITIS

CASE	AGE	SEX	SERUM CALCIUM MG. PER 100 C.C.	CASE	AGE	SEX	SERUM CALCIUM MG. PER 100 C.C.
1	27	F	10.2	26	30	F	9.4
2	48	F	10.2	27	34	F	9.6
3	55	F	9.4	28	24	F	9.2
4	51	F	10.1	29	21	F	10.2
5	36	F	9.4	30	25	F	9.8
6	54	F	11.0	31	49	F	9.5
7	44	F	9.8	32	26	F	11.4
8	25	F	10.5	33	28	F	12.1
9	54	F	10.6	34	28	F	10.4
10	32	F	9.8	35	50	F	10.9
11	31	M	10.4	36	61	F	11.1
12	31	F	10.6	37	37	M	12.2
13	48	F	9.6	38	52	F	10.2
14	10	F	9.6	39	53	F	10.4
15	50	F	10.0	40	36	M	10.7
16	58	F	9.6	41	27	F	10.7
17	33	F	9.4	42	68	M	9.4
18	34	M	11.9	43	61	F	10.2
19	64	F	10.8	44	46	M	10.4
20	58	F	10.6	45	36	F	10.2
21	51	F	10.5	46	47	F	9.4
22	28	F	10.7	47	50	F	10.1
23	27	F	10.0	48	51	F	10.2
24	40	M	9.2	49	21	F	9.6
25	41	F	9.3	50	55	M	10.4

c.c. The average age of this group of patients was forty-one years, and 84 per cent were women.

The serum calcium of fifty cases of osteoarthritis is given in Table II. The mean and standard deviation of this group was 9.986 ± 0.616 mg. per 100 c.c. This group of patients was older, for the average age was fifty-two years. Eighty per cent were women.

TABLE II

THE SERUM CALCIUM IN OSTEOARTHRITIS

CASE	AGE	SEX	SERUM CALCIUM MG. PER 100 C.C.	CASE	AGE	SEX	SERUM CALCIUM MG. PER 100 C.C.
1	40	F	10.0	26	68	F	9.5
2	52	M	10.6	27	52	F	10.6
3	41	F	9.5	28	49	M	9.6
4	58	F	9.3	29	48	F	11.3
5	52	F	9.5	30	50	M	10.6
6	27	M	9.8	31	61	F	9.9
7	53	M	10.8	32	63	M	11.4
8	64	F	9.6	33	62	F	9.9
9	56	F	10.9	34	35	F	9.6
10	62	F	10.6	35	58	F	10.4
11	31	M	9.6	36	52	F	9.8
12	57	F	9.8	37	58	M	11.2
13	48	F	9.2	38	57	F	9.2
14	50	F	9.8	39	63	F	9.8
15	55	F	10.9	40	48	F	9.7
16	46	F	10.8	41	45	F	9.6
17	46	F	9.5	42	49	F	9.7
18	60	F	10.2	43	42	F	9.3
19	46	F	9.7	44	74	F	9.8
20	50	M	11.1	45	69	F	9.8
21	53	F	9.5	46	56	F	9.1
22	45	F	9.8	47	39	F	9.7
23	59	F	10.2	48	53	M	9.1
24	53	F	10.1	49	52	F	10.6
25	53	F	9.0	50	52	F	10.3

TABLE III

SERUM CALCIUM

TYPE OF CASE	NUMBER OF CASES	MG. PER 100 C.C.			
		MEAN	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	PROBABLE ERROR OF STANDARD DEVIATION
Control					
Greene and Boothby	852	10.241	±0.015	0.647	0.010
Control, New York Post-Graduate Hospital					
Hospital patients	50	10.274	±0.060	0.622	0.042
Private patients	128	10.231	±0.046	0.777	0.033
Rheumatoid arthritis	50	10.218	±0.067	0.699	0.048
Osteoarthritis	50	9.986	±0.059	0.616	0.042

The statistical analysis of these two groups of patients, together with the control series, is given in Table III. Greene and Boothby[20] found the mean serum calcium of their control group to be 10.241 ± 0.647 mg. The figures for

the control group from this laboratory give almost identical values, namely, 10.274 ± 0.622 mg. per 100 c.c. Using these figures it may be said that approximately 68 per cent of control cases have a serum calcium between 9.6 and 10.9 mg. and 95 per cent between 9.0 and 11.5 mg. per 100 c.c.

DISCUSSION

The changes in the serum calcium in arthritis are not marked, for of the whole group of 100 cases studied, the values for the serum calcium were outside of this normal range of 9.0 to 11.5 mg. per 100 c.c. in only three cases. There was no evidence that hyperactivity of the parathyroid glands was a factor in the production of arthritis. The mean serum calcium of the cases of rheumatoid arthritis was 10.218 ± 0.699 mg. This value is practically the same as that found in the control cases.

In osteoarthritis the mean was 9.986 ± 0.616 mg. This value is significantly lower than that found in the control group, the difference between the means being 3.4 times the probable error of the difference. We may, therefore, say with assurance that while individual cases of osteoarthritis show a serum calcium within the normal range, the trend is downward and that in this series of cases the mean value was significantly lowered. The difference between the serum calcium level in cases of rheumatoid arthritis and that in osteoarthritis is further evidence that these two conditions represent different clinical entities.

Our present knowledge of the etiologic factors in osteoarthritis does not afford an explanation for the tendency toward a lowered serum calcium in this condition. The decrease may be due to a fundamental change in the calcium metabolism either as a result of the local lesions or as part of a general constitutional reaction. Before changes in the serum calcium can be considered the specific result of the disease process, however, the influence of various associated factors which may have an effect on calcium metabolism must be excluded.

Age is such a factor, for patients with osteoarthritis usually belong to a somewhat older age group than do those with rheumatoid arthritis. Greisheimer, Johnson and Ryan[23] reported that in adults the serum calcium decreased slightly with age. Also a considerable proportion of older individuals show a decrease in the acidity of the gastric juice. This hypochlorhydria or achlorhydria may reduce the absorption of calcium from the intestine either as a result of changes in the reaction of the intestinal contents or in the permeability of the intestinal wall. Many patients with arthritis are anemic. There is a tendency for the serum calcium to be reduced in anemia either as a result of the anemia or because of a concomitant reduction in the serum proteins.

These and probably numerous other factors may be of importance in producing the slight reduction in the serum calcium in osteoarthritis. At the present time no single factor can be accepted as responsible for the changes noted and much further information is needed before the relative importance of each can be determined.

SUMMARY

The values for the mean and the standard deviation of the serum calcium in a group of 50 cases of rheumatoid arthritis were 10.218 ± 0.699 mg. per 100 c.c. These values were essentially the same as those found in control cases

The values for the mean and the standard deviation of the serum calcium in a group of 50 cases of osteoarthritis were 9.986 ± 0.616 mg. per 100 c.c. The changes in the serum calcium in any individual are perhaps without direct clinical significance, but the trend in the group represents a statistically significant reduction in the mean calcium level. The reason for this change is not obvious. Its presence in osteoarthritis and absence in rheumatoid arthritis are further evidences that these two conditions represent separate clinical entities.

This study of the serum calcium furnishes no evidence that hyperactivity of the parathyroid glands is a factor in the production of arthritis.

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OCCLUSION OF THE PORTAL VEIN*

An Experimental Study With Its Clinical Application

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OUR interest in experimental occlusion of the portal vein arose accidentally, in the course of a study of so-called "liver deaths," as one phase of which we endeavored to produce necrosis of the liver by various forms of interference with its blood supply. In this endeavor we failed, chiefly because all the animals in which complete occlusion was done in a single stage died within a few hours, before such changes could possibly ensue. Our interest in the subject was increased by the appearance, during these experiments, of Elman and Cole's paper on the cause of death in portal occlusion, and we determined to undertake a similar investigation, the results of which, together with their apparent clinical application, we are reporting herewith.

HISTORICAL DATA

Experimental ligation of the portal vein was first attempted in 1856 and the clinical implications of such a procedure have been recognized for many years, but so little has been written on the subject that it is almost necessary to begin any discussion of it with a brief review of the literature. Much of the historical data which follow are taken from the excellent survey by Neuhof which appeared in 1913.

Oré, in 1856, first demonstrated that ligation of the portal vein in rabbits results in prompt death, his experiments being corroborated on dogs by Claude Bernard in 1858 and by Schiff in 1863. Bernard attempted no explanation of the fatality until 1877, when he advanced the idea that death was caused by exsanguination, which in turn was due to a stasis of blood in the gastrointestinal tract. He apparently overlooked the experimental proof adduced by Tappenheimer five years before, which we ourselves have just confirmed, that more blood can be withdrawn from a dog by venesection without fatal issue than is accumulated in the gastrointestinal tract after portal ligation. Schiff's explanation that death is due to suppression of liver function is no longer tenable, since it has been repeatedly proved that the existence of an Eck fistula is not incompatible with life.

In 1875 Solowieff proved experimentally that animals can survive the ligation singly, at intervals of five to six days, of the superior mesenteric, the gastrohepatic and the portal veins, and Neuhof, in 1913, confirmed his findings, al-

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though Ito and Omi did not in the single experiment which they reported in 1901. Kusnetzow's demonstration in 1900 that ligation of the portal vein below the entrance of the gastrohepatic vein is fatal, whereas occlusion above this point is not fatal, was not confirmed by any of the last-mentioned workers. Neuhof, however, proved that gradually induced occlusion of the portal vein is not incompatible with life, and pointed out that under such circumstances there are no changes of consequence in the liver because of the development of a collateral circulation in the gastrohepatic omentum.

The extensive experiments in portal occlusion reported from various Russian clinics are not entirely in accord with the results of the experiments just outlined. Roger, for instance, was able to ligate the portal vein for three hours in a dog and half an hour in a rabbit without serious damage, and Duchinowa, in a large number of cases, found it possible to compress the portal vein for thirty-five minutes with no apparent ill effect. Moreover, the blood studies reported by Tschernikoff and his coworkers were done in many cases on animals subjected to repeated compression and decompression of the portal vein.

Elman and Cole, writing in 1934, conclude that in portal occlusion death can be explained entirely on the basis of a loss of blood from the systemic into the portal area of such magnitude that the blood pressure is reduced to a point incompatible with life and is maintained at this critical level until death occurs. They point out, in support of this hypothesis:

1. That the behavior and appearance of animals subjected to portal occlusion corresponds in all respects with the behavior and appearance of animals who die after blood loss due to frank hemorrhage.

2. That the use of transfusion after portal ligation elevates the blood pressure temporarily and postpones death, even though it cannot avert it.

3. That the same results are achieved by ligation of the aorta above the celiac axis, which prevents blood from entering the splanchnic area.

4. That the engorged intestinal tract cannot aid in supplying loss of fluid, as it can in shock due to other causes.

To consider the question in its clinical aspects, Gintrac, in 1857, collected six cases of pathologic portal occlusion, and a considerable number of such cases have since been reported. The number, furthermore, is undoubtedly greater than is apparent from the literature, partly because, as always happens, many cases are not reported, partly because others are included in papers dealing with other subjects. We recently found, for instance, in a publication by C. H. Peck on pain in the upper abdomen and chest, the report of a case of complete portal stenosis for which operation had been undertaken on a mistaken diagnosis of perforated duodenal ulcer, the patient being alive and well eight years later.

Ransahoff, in 1908, noted that even temporary constriction of the portal vein during operations on the gall ducts causes a sharp and prompt fall in blood pressure, and concluded that if the pressure were continued, the phenomena attending portal ligation in animals would undoubtedly be reproduced. Villard reports the loss of a patient because of temporary pressure on the portal vein with a tampon during an operation for biliary disease. On the other hand

Krymholz reports a personal case in which he compressed the portal vein for eight minutes to check hemorrhage in an incised wound of the liver, and Colp reports that in the cases in which he attempted portal ligation as a deliberate therapeutic procedure for pylephlebitis of appendiceal origin, tentative compression of the vein, with subsequent occlusion, had no immediate untoward results, although all the cases were eventually fatal. The clinical and experimental observations are at variance in many respects, but it is safe to state categorically that all the clinical evidence goes to prove that gradual occlusion of the portal vein as the result of some pathologic process is not the same as abruptly produced experimental occlusion.

EXPERIMENTAL DATA

All our experiments were performed under the same controlled conditions. Dogs were selected for use only after the competency of their renal function had been established, and they were prepared for sixty hours; they were given only milk during the first twenty-four hours and only water during the second twenty-four hours, and they were deprived of all food and fluids during the final twelve hours. They were weighed immediately before operation, and they were anesthetized either with intravenous veterinary nembutal (kindly supplied to us by the Abbott Laboratories) or a light ether narcosis.

Series I.—A. Rapid evisceration was done through a midline incision, and the eviscerated mass, which consisted of the entire gastrointestinal tract from the cardiac end of the stomach to the anus, together with the pancreas and the spleen, was ligated, to guard against loss of blood and intestinal contents. This mass was then weighed, to determine its relation to the body weight of the animal. The average in seven dogs was 6.87 per cent.

B. Through a midline incision an aluminum band was placed on the portal vein just below its bifurcation. Seven dogs were used. Profound shock was promptly evident in them all, and death followed in from thirty-three to one hundred thirty-four minutes, the average duration of life being eighty-seven minutes. Immediately after death evisceration was done, by the method just described, and the eviscerated mass was weighed and its weight checked against the body weight. The proportion ranged from 7.98 per cent to 10.9 per cent, the average being 9.92 per cent, in contrast to the average normal proportion of 6.87 per cent. The loss of blood into the intestinal tract, therefore, varied from 1.11 per cent to 4.03 per cent of the body weight, with an average of 3.05 per cent in the seven dogs.

In all cases there was an insignificant rise in the leucocyte count after operation, but no other changes in the total and differential blood count or in the blood chemistry. The clotting time showed a decrease of from ten to thirty-five seconds within the first half hour after operation, a finding which we shall discuss in more detail later.

C. In one dog portal occlusion was done by the method described, following which 685 c.c. of glucose solution were given intravenously. This dog lived for three hours and ten minutes and was the only animal subjected to portal occlusion which was able to stand up after operation or which showed any recovery from

his shock. Such a measure, like the transfusions reported by Elman and Cole, can evidently postpone death temporarily if it is promptly invoked. At autopsy this dog had 250 c.c. of fluid in the peritoneal cavity, a finding which was noted in no other animal in the series.

D. A control series of dogs were bled from 4 to 5 per cent of their body weight, an average of 4.56 per cent. In some instances the venesection was done in one stage, in others it was spaced, to correspond with the average duration of life after portal occlusion. In no case was marked shock evident, and in no case did death follow the procedure. Another dog, however, which was bled 5.8 per cent of his body weight at one time, exhibited profound shock and died within ninety minutes.

Series II.—A. Two dogs subjected to occlusion of more than two-thirds of the portal vein in one stage died within three and a half hours and eighteen hours, respectively.

B. One dog was subjected to occlusion of the right portal vein, followed within a week by occlusion of the left portal vein. A second dog was subjected to occlusion of the left portal vein, followed within ten days by occlusion of the right portal vein. Both dogs died within two hours of the second operation.

C. In one dog half of the portal vein was occluded, then three-quarters, and then the entire vein, at intervals of five to seven days. In the second dog occlusion of two-thirds of the vein was done at the first operation and the occlusion was completed a week later. In neither animal was there any evidence of shock and both were to all appearances perfectly normal when they were sacrificed.

Five weeks after the last operation the abdomen was opened and the portal vein was injected below the point of obstruction, in one case with lead chromate, in the other with a solution of carmine treated with ammonia in gelatin. In both animals an abundant collateral circulation could be traced through three distinct venous channels:

1. Gastric to esophageal.
2. Duodenal and colic to left renal.
3. Inferior mesenteric to hemorrhoidal to hypogastric.

Series III.—Simultaneous ligation of the portal vein and of the gastric, splenic, and inferior and superior mesenteric arteries, with the idea of preventing part of the blood from entering the portal system, was followed by death within ninety minutes. Elman and Cole, in a similar experiment, were able to keep their animals alive more than six hours by the use of transfusion, and noted that less blood was required to achieve this result than when arterial ligation was not done.

From these three groups of experiments we have drawn the following conclusions:

1. Occlusion of the portal vein in a single stage is invariably fatal, as is successive occlusion of the portal branches or the occlusion of more than two-thirds of the vein at any one time.

2. Stage occlusion, as Neuhof originally proved, is not incompatible with life because this method permits the development of an abundant collateral circulation which takes over the task of the portal circulation.

3. The explanation advanced by Elman and Cole for the cause of death in portal occlusion, that the loss of blood into the gastrointestinal tract produces a fall in the blood pressure to a point incompatible with life, is not acceptable. That the blood loss plays an important part in the fatal issue cannot be denied, but that it is the only cause of death, or even the principal cause, we do not believe, for we have proved, as Tappenheimer proved in 1872, that in order to cause death, the animals must be bled by venesection far beyond the blood loss into the intestinal tract after portal occlusion. Furthermore, we do not believe that the relatively abrupt fall in blood pressure after portal occlusion is likely to be caused by the relatively slow loss of blood into the intestinal tract. Finally, an additional argument against the theory is the fact that the greatest volume of blood which reaches the right heart by way of the inferior vena cava does not come from the liver.

4. Our own theory is that death after portal obstruction is to be explained in two ways, the first explanation having to do with the initial fall in the blood pressure, the second with its maintenance at a level incompatible with life. The initial fall, in our opinion, is brought about by the same neurogenic factor, with the same resulting inhibition, as is present in primary shock, plus the abrupt shunting of a large amount of blood from the general circulation, which implies, in turn, a corresponding decrease in the volume of the circulating blood. The maintenance of the blood pressure at the critical level is also due to two things. In the first place, there is a continued gradual withdrawal of more and more blood from the general circulation into an area from which it cannot escape, with a corresponding gradual decrease in the volume of the circulating blood; in the second place, there is a stagnation in the intestinal tract of the blood thus diverted, which has the same effect as the loss of the same amount of blood would have in any other hemorrhagic condition. The neurogenic factor, it seems to us, is amply established by the experiments of Thole, who demonstrated that when the vagus nerve is sectioned before portal occlusion is done, the drop in the blood pressure is more gradual and the duration of life is longer, though death is just as inevitable. The decerebration experiments of various other Russian investigators are confusing rather than helpful, it seems to us, because the procedure of decerebration in itself introduces factors which hopelessly cloud the issue.

5. The decrease in the clotting time of the blood which we observed in our experiments is entirely in keeping with the changes reported by Russian investigators following portal ligation. The decrease is more notable terminally than initially, but we did not carry our studies beyond the first half hour after operation, because their work is so complete. In all the cases which they report the decrease was very marked; in one case reported by Tschernikoff and his coworkers, for instance, the clotting time fell from three minutes ten seconds before operation to fifteen seconds within eighty minutes after operation. There was al-

ways a prompt return to normal figures when only temporary ligation was done, but the longer the compression had endured the slower was the return to normal.

Why this decrease should occur is not clear. The original observation was made accidentally, when it was noted that after portal occlusion the cannulas in use promptly became choked with clotted blood. Liver changes do not seem responsible, for death occurs too promptly after operation for the liver substance to be seriously affected. The theory that the spleen delivers into the circulation certain products which tend to lower coagulability does not hold, for in some experiments the splenic circulation was tied off before portal occlusion was done. Detailed morphologic and chemical blood studies revealed no significant blood changes. Brandes and Simonds' explanation of a similar decrease in the clotting time after mechanical constriction of the hepatic vein, that the heparin in the blood is used up too rapidly, cannot be accepted here, for the source of the supply of heparin is not altered by portal occlusion. The speculative explanation advanced by Tschernikoff and his coworkers, that some obscure functional disturbance of the intestine is responsible, is at least reasonable, for the effect of the products of intestinal metabolism under such circumstances is undoubtedly very important. Their results are as yet inconclusive, and we propose to continue our own investigations along the line they have suggested and to study the effect upon the blood of lymph obtained from the gastrointestinal tract by way of the thoracic duct.

CLINICAL CONSIDERATIONS

The idea of ligation of the affected vein for pyemia of various origins is no new thing. John Hunter did it successfully on the saphenous vein in 1784, and in the following century many similar attempts were reported, though all of them did not end so well. General interest in the operation, however, was not aroused until Zauful, in 1894, suggested its application to the internal jugular vein in pyemia of otitic origin and Viereck, seven years later, reported 108 such cases with recovery in 89.

The operation was next applied to puerperal pyemia. In 1894 and in 1898 Sippel and Freund had proposed treating this disease by excision of the affected pelvic veins, in addition to hysterectomy, but the mortality of the latter procedure was in itself so appalling that their suggestion won little attention and less approval. In 1902, however, Trendelenburg and Bumm published their results with simple ligation of the pelvic veins in puerperal pyemia, and Miller's comprehensive study in 1917 established beyond doubt the wisdom of this procedure in the occasional properly selected case. The latter's collected statistics show a gross mortality of 51.6 per cent, and a corrected mortality, for properly chosen cases, of 33.9 per cent, and there can be no question that such a reduction in the mortality of a disease which, untreated, exhibits a death rate of 60 to 70 per cent, warrants earnest consideration of the method which has brought it about.

The theoretic possibility of a similar procedure in pylephlebitis of appendiceal origin is obvious, as Neuhof was the first to point out. That appendicitis is the most frequent antecedent disease in this condition is not nearly

so important a consideration as the fact that pylephlebitis is a complication of acute appendicitis in a definite, although small, percentage of all cases, especially in old people, and the fact that it is variously estimated to be responsible for from 5 to 10 per cent of all fatal cases. Moreover, while recoveries after it are recorded, although many of the reported cases leave one with the impression that the cure occurred when the existence of the complication was never proved, the mortality is still enormous. It was 59 per cent in the 53 cases collected by Eliason, who comments, with reason, upon the wide discrepancy between the 20 recoveries in the 23 cases collected by Schlesinger (which are included in his 53 cases) and the 7 recoveries in the 30 cases which he himself collected. It seems scarcely necessary to point out that the latter figures are far more in keeping with the experience of most surgeons than are the former.

In a disease of such gravity heroic measures are undoubtedly justified, but one must be certain, before adopting them, that they are not too heroic. In the first place, the operation is frequently done too late even when it is done immediately, for thrombophlebitis may be coincident with the clinical onset of the disease, as in the case mentioned by Keyes, in which death occurred from this complication four days after the removal of an acutely inflamed but nonsuppurative appendix. In the second place, although the fundamental pathologic process is the same and the fundamental principle of treatment is the same, there is an even wider difference between ligation of the portal vein and ligation of the pelvic veins than between ligation of the pelvic veins and ligation of the internal jugular vein. Venous ligation in an area drained by one vein and a similar procedure in an area drained by many veins are by no means the same thing. In the third place, as we have already pointed out, there is a wide difference between gradual occlusion of the portal vein by a slow pathologic process, and its abrupt mechanical occlusion by a surgical procedure. Finally, and most important, all the experimental evidence and most of the clinical evidence forces one to the conclusion that portal occlusion is either entirely incompatible with life or introduces so serious a degree of risk that the remedy threatens to be even worse than the disease for which it is invoked.

But the remedy has been tried. Beer, following Neuhof's suggestion, undertook deliberate ligation of the portal vein in pylephlebitis of appendiceal origin, and as the first step attempted to anastomose the spermatic vein with a branch of the inferior mesenteric vein, with the idea of increasing the collateral circulation. When the attempt failed he resorted to omentopexy, which was included in Neuhof's original suggestion, and three days later performed cholecystectomy and portal ligation. The patient's jaundice promptly decreased, but he died within forty-eight hours.

In 1926 Colp reported four fatal cases of ligation of the portal vein, one of which can be promptly discarded, since, because of the distortion caused by adhesions, the superior mesenteric vein was ligated in the mistaken belief that the ligature was being placed on the portal vein.

Of the other three patients, one was moribund at operation and died within three hours, the second lived two days, and the third, in whom occlusion was done in two stages, died six days after the second operation. Autopsy in two

cases showed hepatic insufficiency, which the author, probably correctly, attributes to the suppurative process rather than to the operative procedure. In both cases an adequate collateral circulation was present, which means, Colp points out, either that a beginning thrombophlebitis of the portal vein opens up collateral veins, or that the hepatopetal veins in some individuals are competent to take over the circulation immediately when sudden occlusion occurs.

It should be mentioned here that detailed studies of the collateral circulation were made by Charpy in 1898 and by Pick in 1909, the original studies having been made by Frerichs in 1861. Pick reports a particularly striking case in which the portal vein had been occluded for years by a cavernoma of the vessel wall, but in which the portal blood was carried to the liver by veins in the gastrohepatic omentum. The existence of this set of veins was pointed out originally by Charpy, who coined for them the term hepatopetal.

Colp's and Beer's cases, unsuccessful though they were, at least proved that in the human being death does not follow portal occlusion as promptly as it does in the experimental animal, and the same conclusion can be drawn from the cases of ligation of the portal vein for injuries of the liver which were reported by Brewer in 1908 and by Hallopeau in 1910. Furthermore, in pylephlebitis of appendiceal origin conditions are more favorable for portal occlusion than they are in the experimental animal, since there is always present before the occlusion some degree of venous obstruction. It would seem, therefore, on the basis of both clinical and experimental evidence, that if the occlusion has involved a third or more of the lumen of the vein, it might be justifiable to risk the surgical completion of the occlusion. If the occlusion has not progressed to this point, however, or if one branch of the vein is already occluded, the procedure introduces an element of risk even greater than the risk inherent in the untreated disease. In other words, the operation, however sound may be the theory upon which it is based, has necessarily a very limited field, and it would be well for the surgeon who proposes to undertake it to remember that Gerster's dictum, uttered in 1903, still cannot be gainsaid, that while exposure and evacuation of the thrombosed veins is always highly desirable, the procedure is always difficult and is usually impossible.

SUMMARY AND CONCLUSIONS

1. A brief résumé is given of previous experimental and clinical work on portal occlusion.

2. A series of experiments is reported which prove:

a. That complete occlusion of the portal vein in one stage, as well as successive occlusion of both branches, is incompatible with life.

b. That occlusion of the main portal trunk in several stages is a feasible procedure if not more than two-thirds of the vein is occluded at any one time.

3. Experimental proof is advanced to disprove the theory that the loss of blood into the gastrointestinal tract is the only cause or the chief cause of death in portal occlusion.

4. The theory is advanced that death in portal occlusion is due:

a. To an abrupt and immediate fall in blood pressure, brought about by a neurogenic factor with resulting reflex inhibition, as in primary shock, plus the abrupt diversion of a large amount of blood from the circulation, with a resulting decrease in blood volume.

b. To the maintenance of the blood pressure at this critical level, as the result of a continued gradual withdrawal of blood from the circulation, with a continued gradual decrease in blood volume as the diverted blood is collected in an area from which it cannot escape, plus the stagnation of this blood in the gastrointestinal tract, which has all the effect of primary hemorrhage.

5. Occlusion of the portal vein for pylephlebitis of appendiceal origin is discussed in its clinical aspects, and it is concluded that the operation, although theoretically sound and technically possible, actually has a very limited field and always is attended with very grave risks.

NOTE.—This work was done with the cooperation and approval of Dr. Urban Maes, Director of the Department of Surgery of the Louisiana State University, School of Medicine. Our thanks are due to Dr. D. D. Baker, of the Department of Anatomy, who demonstrated the collateral circulation for us, and to Dr. W. B. Wright, of the Interne Staff of Charity Hospital, who assisted us in many of the experiments.

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INFLUENCE OF DIET UPON THE TOXICITY OF ETHYLHYDROCUPREINE HYDROCHLORIDE*

Preliminary Report

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IN A PREVIOUS study† we found that the animals on carrot or mixed diet reacted to the injection of cocaine more or less similarly; though the animals on the carrot diet reacted more vigorously. The animals on water and oats diet exclusively were definitely more sensitive to cocaine poisoning. In general, in the oat-fed animals, the toxic effects of the cocaine were definitely greater and recovery was considerably delayed. In continuation of this study we have undertaken a new series of experiments, presented here, replacing cocaine with ethylhydrocupreine hydrochloride.

The observations were conducted on 55 rabbits, divided into 3 groups. Each group was kept for two to three weeks on special diets. In the first group the animals (15 rabbits) were fed oats, carrots, cabbage, alfalfa and water (as standard normal diet); the second group (20 animals) was fed water and oats, and the third (20 animals) on carrots exclusively. During the experiment the urine of the animals was tested daily for its reaction (Folin's method). At the end of the first week the urine of the rabbits of the first group was slightly alkaline or acid, although more generally alkaline; the urine of the second group (oat diet) showed distinct and stable acid reaction (up to 90); and that of the third (carrot diet) group was persistently alkaline.

The experiments consisted in injections of ethylhydrocupreine hydrochloride into the marginal ear vein of a rabbit. The injections were performed uniformly, the duration of injection being about ten seconds. The dose of ethylhydrocupreine was 18 mg. in 1 c.c. of water per kilo of animal's weight. After the injections the animals were observed for forty-eight hours, and their death or survival recorded. The deaths in the greater majority of cases occurred in from one to three minutes after the injection of the ethylhydrocupreine.

The results of experiments are presented in Table I.

Table I

DIETS	DIED	SURVIVED	TOTAL NO. OF ANIMALS	MORTALITY PER CENT
General diet (first group)	8	7	15	53
Oat diet (second group)	12	8	20	60
Carrot diet (third group)	8	12	20	40

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CONCLUSIONS

1. Animals on an oat diet are definitely more sensitive to the ethylhydrocupreine than the animals on a mixed diet.

2. Animals on carrot diet reacted less severely than the animals on mixed diet.

3. It is quite probable that the differences in reaction of the animals to this alkaloid are due to difference in the acid-base balance of the system, as evidenced by the difference in the urinary reaction.

It is my pleasant duty to express sincerest gratitude to Dr. Bernard Fantus, for his suggestions and criticisms proved of inestimable value.

FAT EMBOLISM*

A Control Study of Blood Serum and Urine

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THE diagnosis of fat embolism in fractures offers many difficulties from a clinical viewpoint. The symptoms of this condition being more or less irregular and somewhat indefinite, depending purely on the local phenomena produced by the liberated fat droplets, leaves the clinician to prove the diagnosis by one of the laboratory methods. As the quantitative fat determinations of the blood are very complicated, and the variations in the normal so great, this method at the present time is of little value. The presence of fat droplets in the sputum, Warthin's sign, is present in numerous other chest conditions so it cannot be considered reliable. This leaves us with but the examination of the urine and blood serum, in these cases, to substantiate such a diagnosis.

As far as we have been able to determine from the literature the study of the urine and blood serum in normal cases, and fresh fractures, has never been done. For this reason the following work has been carried on to see whether or not free fat is found in normal control cases.

Two hundred miscellaneous hospital cases were used for the urine examination. In this series only ambulatory male patients were used because of the greater ease in collecting the urine specimen. Great care was exercised in this work, to have the patient completely empty the bladder, as the authors have shown in a recent article,[1] that unless all of the urine of the bladder is collected, the floating fat droplets will not be expelled, and hence the urine examination would convey the wrong conclusions.

The urine specimens were collected and placed in a cool place overnight. The next morning the surface of the urine was carefully examined for floating

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fat droplets. Any questionable droplets were removed, stained with Sudan III and examined microscopically. In this entire series not one case showed any free fat.

The Serology Department of the Cook County Hospital was kind enough to permit us to have the unlimited use of the blood serums that were sent to them for Wassermann tests. Each specimen was labeled, centrifuged and then placed in the refrigerator overnight. The following morning each tube was carefully examined. All questionable droplets or débris were stained and examined microscopically. On five occasions definite fat droplets were found floating on the surface. However, our findings proved only that the needles had been kept in liquid petrolatum in each case, and evidently the boiling did not dislodge the oil from the inside of the needle. When the blood was drawn the oil found its way into the specimen. Eleven of these cases showed a chylous emulsion which rapidly found its way to the surface. This is physiologic as any blood serum drawn within an hour or two after the ingestion of any fatty material presents this phenomenon. In each of the above cases, the above contention was found to be correct.

Now that we had to our own satisfaction been able to determine what non-traumatic cases showed, we studied fifty fresh fracture cases.

Only male patients, suffering from major fractures, were studied. The duration of the fractures was from one to seven days. The urine was collected by having the patient either in the sitting or standing position, and then having him completely evacuate the bladder. Ten cubic centimeters of blood were then drawn from the arm and both specimens were centrifuged and examined. The urine residue was stained with Sudan III, and examined microscopically. Occasionally casts were found, but in not one instance were we able to find fatty casts such as Riedel[2] described as being present in the urine of 42 per cent of fresh fracture cases.

The casts were no more numerous, nor in any way different, than the casts found in any group of urine specimens.

Not one specimen of blood serum or urine showed any evidence of free fat droplets.

For a résumé of these cases refer to Table I.

TABLE I

Type of Fracture:		*Number of Hours Since Accident:*	
Tibia	20	1- 24 hours	11
Femur	14	24- 48 hours	8
Humerus	7	48- 72 hours	6
Fibula	5	72- 96 hours	9
Patella	1	96-120 hours	6
Forearm both bones	1	120-144 hours	3
Pelvis	1	144-168 hours	7
Ribs	1		
	—	Total cases	50
Total cases	50		
Number of Cases Showing Fat:		*Number of Cases Showing Urine Casts:*	
Urine	0	9 cases from 1-2 to 1-12 L.P.F. in centrifuged specimens.	
Blood serum	0		

CONCLUSIONS

1. Control cases in the study of fat embolism revealed no free fat in either the blood serum or urine specimens.

2. Care should be used completely to empty the bladder of suspected cases, otherwise the floating fat will escape expulsion, if present (Jirka and Scuderi).

3. All questionable films and débris should be stained with Sudan III for final classification.

4. We were unable to confirm the findings of Riedel, that fatty casts were frequently found in the urine of fresh fracture cases.

5. Needles kept in oily substances should never be used for blood collection, if the results are to be relied upon.

The fracture cases are from the service of Dr. Wm. R. Cubbins. The authors wish to express their gratitude.

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LOCAL ADRENALIN EFFECT AFTER SYMPATHECTOMY*

I. The Peripheral Vessels: A Preliminary Report

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THE increased sensitivity to adrenalin which certain tissues acquire after those tissues have been deprived of their sympathetic nerve supply is known.

The best example is probably that sensitization which occurs in the denervated heart as shown by Elliot in 1905.[1] Auer and Meltzer had, in 1904,[2] remarked the increased sensitiveness of the denervated iris. Anrep[3] demonstrated that denervation causes the blood vessels in the ear of a rabbit to respond to dilutions of adrenalin as high as 1 to 250 million, a phenomenon which may be put to use in the assay of small quantities of adrenalin. Cannon[4] has used an isolated strip of intestinal muscle to assay and detect the presence of adrenalin in the blood. Recently Freeman, Smithwick, and White[5] reported evidence of sensitization of peripheral vessels in human patients who have been sympathectomized for Raynaud's disease. They used intravenously administered adrenalin or endogenous adrenalin secreted in insulin hypoglycemia.

During the course of some experiments in this laboratory on sympathectomized dogs, adrenalin was injected subcutaneously in a sympathectomized area

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of two dogs. Dry necrosis occurred at the site of injection in both. One of these dogs died. The occurrence of abscess and gangrene is not an uncommon complication of adrenalin administration intramuscularly or subcutaneously in animals and in man, and is usually attributed to individual idiosyncrasy or infection. However, in the case of these two dogs, the possibility exists that the pathologic changes might have been caused by a sensitivity of so marked a degree that adrenalin in the concentrations given causes such a prolonged and pronounced vasoconstriction as to result in ischemic necrosis. This being the case, the increased sensitivity demonstrated by the above mentioned authors is of pathologic as well as of pharmacologic and physiologic interest. It was decided, therefore, to study this phenomenon under stricter experimental conditions than those which governed in the case of these two dogs.

Four animals were prepared by bilateral lumbar sympathectomy. Thus the arteries of the hind legs were denervated of their autonomic supply.

One cubic centimeter of 1 to 1,000 adrenalin solution was injected into the subcutaneous tissues of the hind legs of two of these dogs, and 0.5 c.c. of 1 to 1,000 adrenalin solution was similarly injected into the hind legs of the other two dogs. Aseptic precautions were taken. As controls, the same quantity of physiologic saline solution was injected into a nearby area within the sympathectomized region of each of the four dogs and the same quantity of adrenalin was injected into the subcutaneous tissues of one of the forelegs, which were of course normally supplied with sympathetic nerve fibers. All the needles and syringes used were sterilized and handled under the same conditions.

In every instance where the adrenalin was injected into the sympathectomized leg, a definite area of necrosis developed within twenty-four hours. At the onset all these areas of necrosis were typical. They were circular, sharply demarcated, and dry. On about the third day separation of the necrosed tissues occurred, and in two animals the necrosis later became extensive. The appearance of this late necrosis suggested that adrenalin had seeped to a more dependent level producing necrosis 3 to 5 inches below the point of injection. In none of the other sites of injections for control, saline or adrenalin, was there any evidence of disturbance at any time after the injection.

All these animals were injected after a period of at least eight days had elapsed following sympathectomy. The time at which this sensitization occurs has not been accurately determined. Freeman, Smithwick, and White state that a latent period of six to eight days must pass before the sensitization is manifested in the peripheral arteries. In the case of the iris and isolated intestinal muscle the sensitization seems to be immediate. In one other dog adrenalin was injected at the time of operation and twenty-four hours later. The dog died in forty-eight hours from pneumonia and at that time there were no evidences of gangrene, showing in this case at least, the sensitization does not occur in the first twenty-four hours. This method of determining the time of sensitization has been abandoned and a different and more delicate type will have to be used. We are also investigating the possible relation between this phenomenon and the changes which occur in certain functional vascular disorders.

The finding is reported here for two reasons: Sympathectomy has become a more or less popular surgical procedure for a variety of conditions. The danger in injecting adrenalin into the arm of a patient who has had a cervical sympathectomy is a real one.

Gask, in a recent issue of the *British Journal of Surgery*,[6] casts some doubt on the uniform excellence of results heretofore claimed for sympathectomy in Raynaud's disease. In following cases for over a year in which there had been an immediate and dramatic improvement at operation, he noticed a return of symptoms. Inability of the organism to adjust for heat and cold through the vascular system may not be the whole explanation.

SUMMARY AND CONCLUSIONS

1. Tissues supplied by nerves from the thoracicolumbar division of the autonomic nervous system when deprived of these nerves tend to become hypersensitive to adrenalin. This is true of the iris, the heart, intestinal musculature, and is now shown to be true in peripheral vessels.

2. Peripheral tissues, when the arterial supply is sympathectomized, undergo necrosis on the injection of ordinary doses of moderate dilutions of adrenalin subcutaneously, in the dog.

3. The time of the onset of sensitization and the possible relation to peripheral vascular disease is being studied.

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THE FUNGICIDAL POWER OF PHENOL DERIVATIVES*

II. Strength in the Presence of Proteins

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IN AN earlier article[1] we reported the results of a series of studies made of the effect on the fungicidal power of certain phenol derivatives of the introduction of various alkyl groups and halogens. It has long been recognized that there is often a wide difference between results obtained in the test tube and successful clinical application. Of the possible factors responsible for this difference two are perhaps preeminent; i.e., first, the physical difficulty of delivering an effective quantity of a given drug to the site of the infection; second, a tendency on the part of the drug applied to combine more freely with the epidermal structures present than with the organisms growing therein. In other words, assuming a certain amount of penetration on the part of the remedy applied, there still remains the requisite of ability to kill or restrain in the presence of tissue proteins completely surrounding the invading organism.

The present work was undertaken in the hope of gaining some information concerning the activity of a group of drugs when used in the presence of certain proteins. For this three of the more active phenols were compared with iodine and four other drugs of common clinical usage. The choice of proteins to be used offered certain difficulties. After considerable preliminary work those chosen consisted of human vesicle fluid, blood serum, and hide dust. The vesicle fluid was obtained from vesicles induced by cantharides plasters, the fluid being withdrawn aseptically and kept in sterile receptacles. Blood was obtained in the usual manner and the supernatant serum decanted. The hide dust was the ordinary commercial product employed by leather manufacturers in the titration of tannic acid solutions. According to information relative to its preparation the keratin and keratohyalin elements present are preserved sufficiently intact to serve the purpose of these estimations. Its use in preference to that of human scales, as in the work of Legge, Bonar and Templeton[2] was prompted by a desire to demonstrate any possible presence or absence of selective action on the part of drugs given the opportunity of reaching both organism and protein in comparable concentration.

PROCEDURE

Ten cubic centimeter portions of broth suspension of *Monilia tropicalis* which had been growing for seventy-two hours at 37.5° C. were centrifuged for five minutes at 1,000 revolutions per minute. After being centrifuged the yeast

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was found to be packed into a volume of about 0.05 c.c. The broth was drawn off with a pipette. To one tube was added enough vesicle fluid to make a total volume of 10 c.c., to a second tube was added a similar volume of blood serum, and to a third tube a like amount of distilled water. The tubes were shaken until homogeneous suspensions were obtained.

Tests were made by placing 0.5 c.c. of organism suspension in each of a series of sterile tubes and adding to each, 1 c.c. of the particular drug solution being used. At the end of fifteen-minute intervals transfers were made to broth tubes which were incubated for seventy-two hours before readings were made. Tests were made in the above manner with the organisms suspended in distilled water (control), in blood serum, in vesicle fluid, and also with the original broth suspension.

Hide powder, being a solid, had to be handled in a slightly different manner. Portions of hide powder (0.075 g.) were weighed into each of a series of sterile test tubes. It was found to be very difficult to sterilize the hide powder and still have a material left which could later be put into a more or less homogeneous suspension. This was finally accomplished by heating the tubes containing the hide powder at 55° C. for three separate periods of two hours, one and one-half hours and thirty minutes, respectively.

When tests were made 1 c.c. of a distilled water-organism suspension was added to each tube of hide powder. The resulting suspension was stirred with a transfer needle in order to obtain as near a homogeneous suspension as possible. Then 2 c.c. of drug were added and tests made as before.

The amounts of solid material in each test solution were approximately the same. The vesicle fluid was found to contain 0.075 g. of total solids per c.c.; cow blood serum, 0.087 g. per c.c.; and the broth solution 0.065 g. per c.c. The hide dust was weighed out so that each cubic centimeter of test suspension contained approximately 0.075 g.

DISCUSSION

The results of the tests are shown in Table I. In it are noted the type of suspension and the highest dilution of the drug effective against a particular suspension. Where growth occurred even in the presence of a saturated solution of a given drug, it is shown by the word "none." The approximate dilution representing saturation of such drugs is given in a footnote at the bottom of the table.

Generally considered the results noted in the table reemphasize the possible discrepancies that may exist between promising laboratory experimentation and successful clinical results. As noted in the previous paper[1] iodine surpasses all other drugs tested when used against water and broth suspensions. Its power to kill, however, is greatly reduced in the presence of proteins. An effectiveness up to 1:75,000 in water suspension and 1:20,000 in broth suspension is successively reduced to 1:5,000, 1:2,000 and 1:1,000 in the presence of hide dust, vesicle fluid, and blood serum, respectively. N-hexyl resorcinol, on the other hand, while less effective on water and broth suspensions is not so drastically effected by the introduction of proteins. Compared to an effective dilution of

1:20,000 and 1:15,000 in water and broth suspensions, it retains its effectiveness in dilutions of 1:5,000, 1:2,000, and 1:2,000 in the presence of the three substances used. Not only is its relative diminution less than that of iodine, but its effective dilution surpasses that of iodine in blood serum. Chlorothymol, while moderately active in the presence of water and broth, fails to prevent growth in a saturated solution under the conditions imposed by the other tests. Thymol,

TABLE I

COMPARATIVE STUDY

ORGANISM: MONILIA TROPICALIS. TIME: 15 MINUTES

DRUG	WATER SUSPENSION (EFFECTIVE DILUTION)	BROTH SUSPENSION (EFFECTIVE DILUTION)	HIDE DUST SUSPENSION (EFFECTIVE DILUTION)	VESICLE FLUID SUSPENSION (EFFECTIVE DILUTION)	BLOOD SERUM SUSPENSION (EFFECTIVE DILUTION)
Iodine	1:75,000	1:20,000	1:5,000	1:2,000	1:1,000
N-hexyl resorcinol	1:20,000	1:15,000	1:5,000	1:2,000	1:2,000
Chlorothymol	1:9,000	1:8,000	None*	None	None
Thymol	1:2,000	1:2,000	1:1,250	None	None
Sodium hypochlorite	1:7,500	1:2,500	1:750	1:500	1:500
Salicylic acid	1:1,250	1:1,100	None	None	None
Benzoic acid	None*	None	None	None	None
Sodium thiosulphate	None	None*	None	None	None

*"None" indicates that even a saturated aqueous solution was not strong enough to kill the organisms.
Approximate dilution representing saturation:
Chlorothymol, 1:5,000
Thymol, 1:1,100
Salicylic acid, 1:500
Benzoic acid, 1:350
Sodium thiosulphate, 1:2

the third member of the series chosen for these modified tests, while outranked by the preceding, retains a power to kill in the presence of hide powder, but loses this power, even in a saturated solution, in the presence of vesicle fluid and blood serum.

Of the four commonly used drugs, presumably salicylic acid and benzoic acid enjoy a popularity of greatest duration. Under the conditions imposed, salicylic acid appears to act only in high concentration in the presence of water and broth suspensions, and fails entirely even in saturated solution in the presence of the other three substances used. Even more striking is the complete inability of benzoic acid to kill in saturated solution under any of the conditions imposed by these experiments. The same is to be said of sodium thiosulphate, though its solubility is much greater. Apparently, so far as these tests are indicative, the recommendations of Osborne[3] regarding sodium hypochlorite are well founded. While a greater concentration is necessary, it retains an ability to kill throughout the series of tests.

Results obtained under the conditions of these experiments reveal that fungicidal tests made in water and broth suspensions may be valuable for making comparisons between various drugs in the laboratory but can give only a rough idea as to the manner in which they will react in actual clinical trial.

The need for a standardized method of testing in the presence of proteins is indicated. The authors have used egg white as an easily obtained protein material for testing, but attempts to standardize its effect against that of blood

serum and other natural protein solutions were only partially successful. Better results were had by using commercial peptone mixtures. Further work along this line will be done later.

The results also seem to show that some of the higher phenol derivatives deserve more clinical study in the treatment of fungus diseases than they have had thus far.

SUMMARY

1. Three phenol derivatives have been compared with iodine and four other drugs of common clinical usage in the presence of blood serum, vesicle fluid, and hide powder.

2. The fungicidal power of iodine as determined in water and broth suspensions was greatly reduced in suspensions of serum, vesicle fluid, and hide powder; that of n-hexyl resorcinol was reduced but the relative diminution was less than that of iodine; saturated solutions of chlorothymol failed to kill in the presence of proteins while thymol was effective only in the presence of hide powder; benzoic acid, salicylic acid and sodium thiosulphate were entirely ineffective in the protein suspensions, but sodium hypochlorite maintained its killing power in high concentrations.

3. Higher phenols have been found worthy of further clinical study in the treatment of fungus diseases.

4. A drug more specific in its action than those now available is needed.

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LABORATORY METHODS

THE PRESERVATION OF BLOOD FILMS*

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THE preparation and preservation of blood films for microscopic study is important. Canada balsam plus a suitable cover glass has been the approved method. Slides thus preserved are usually commendable from the microscopist's viewpoint, providing the original staining technic is good. The handling of balsam, cover glasses, and the period required for drying the mounted film is time consuming.

NEW METHOD

We have substituted a technic which will enable one to quickly render a film permanent after staining. It is cheaper and the time required for the permanent preservation of one or more slides is a matter of a few seconds.

Fig. 1.

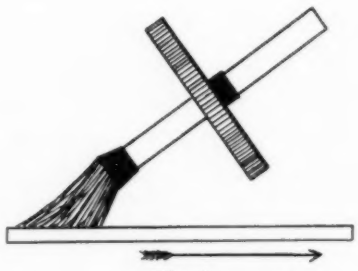

Fig. 2.

Fig. 1.—Commercial glass jar with Sable brush soldered in place. This is an air-tight receptacle for lacquer.

Fig. 2.—Method of coating the blood film.

Technic.—This consists of the application of a thin coating of lacquer† by means of a Sable brush‡ to the surface of a clean, dry, prepared blood film which has been stained. If the lacquer on prolonged standing becomes cloudy, its transparency is readily restored by the addition of a small quantity of transparentizer. This is always available from the lacquer supply firm.

Apparatus.—A commercial glass jar with an air-tight cover in which the brush may be mounted permanently is recommended as a container for the

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†The lacquer is a commercial product of the J. H. Weil Company, 1315 Cherry Street, Philadelphia. (Lacquer Number 14.)

‡The Sable Brush, Number 16, may be obtained from any art supply company.

lacquer.* This is illustrated in Fig. 1, while in Fig. 2 the method of application of the lacquer to the horizontal film is demonstrated.

Originally in this work an attempt was made to use a collodion film, but this was found to distort the red blood cells so that it was abandoned. The

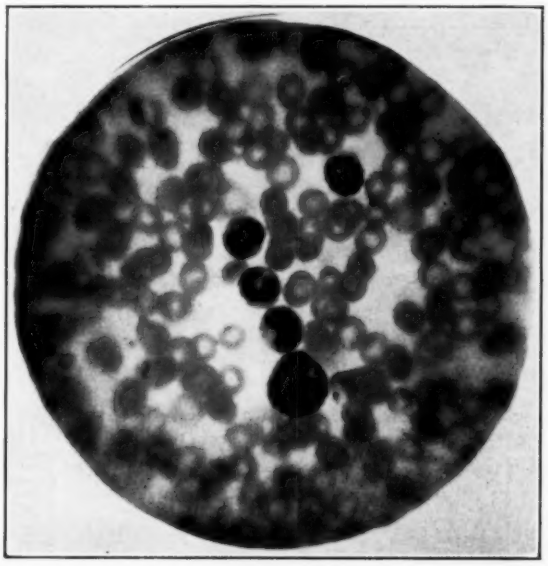

Fig. 3.—Photomicrograph of a blood smear of chronic myeloid leucemia with a 2 mm. apochromatic objective and oil immerson. Wright's stain and preserved under lacquer for three months.

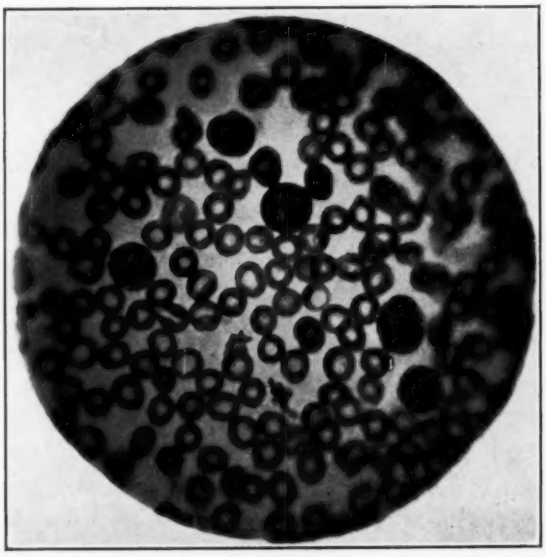

Fig. 4.—Photomicrograph of a blood smear of chronic myeloid leucemia with a 2 mm. apochromatic objective and oil immersion. Wright's stain and preserved with balsam and a cover glass for three months.

lacquer has been found to preserve the original appearance of the red blood cells with but slight distortion. Even with damp weather the lacquer dries

*The glass jar is stocked by any photographic supply company and is manufactured by a New York specialty firm.

within seventy-five seconds after application. It is not affected by either xylol or cedar oil when dry. It probably has a refractive index approximately that of glass which is usually 1.5.

Observations of slides permanently preserved with lacquer were made with a Zeiss binocular microscope, utilizing 10X oculars and a 20 mm. dry objective or a 2 mm. apochromatic oil immersion objective. Under the dry objective the film has an appearance superior to that of the unmounted slide. The nuclei of the cells are particularly distinct. With the oil immersion objective the film is equal in refractiveness to one mounted in balsam and a cover glass. Freyfeld's granules and Doehle's inclusion bodies, as well as other cytoplasmic or nuclear structures, are well detailed.

Those who may wish to use this method are cautioned to have a dry slide and to apply the lacquer with one stroke of the brush. It is desirable to obtain a thin smooth application of the lacquer. This need not be performed with excessive rapidity, but the brush should not carry an excess of the lacquer. Wiping the brush on the rim of the glass jar before touching the slide is recommended.

We wish to thank Dr. Frederick Kramer and Mr. Joseph Poppel for their aid in the preparation of the illustrated apparatus.

THE USE OF RAZOR BLADES FOR TISSUE SECTIONING*

I. A. Nelson, M.D., Tulsa, Okla.

RAZOR blades may be used in sectioning tissues with either freezing or paraffin microtomes without the use of special razor blade clamp holders. To use a razor blade one merely needs an old discarded regular microtome knife, or a piece of metal sharp on one edge (similar to a microtome knife) and some cement. This laminated assembly seems to diminish quivering.

Good support of the cutting edge is essential to success in using razor blades. With a heavy holding knife, or its equivalent, which has a sufficient hollow grinding, blades that have a long bevel may be so placed that the hollow sharpened edge of the holding knife makes intimate contact with the long bevel very close to the edge or the second short cutting bevel. Some blades have two bevels.

We have used the Schick razor blade for over five years in a modified Spencer razor blade clamp holder, and found it the best in quality of steel, in bevel, and in rigidity for frozen and paraffin sections. Recently we wanted to cut blocked tissues by frozen method but our blocks were too large for the effective area covered by a Schick blade in a Spencer freezing microtome. We found that either a Sextoblade blade or a Durham Duplex blade could be used by gluing to an old knife if we provided support for the bevel. This support can be obtained by using sufficient glue to act as a filler in the space between

*From the Pathology Laboratory of St. John's Hospital.
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the blade and the holder to keep the long bevel in contact with the cutting edge of the old regular microtome knife. The Sextoblade must have the back snapped off and the Durham Duplex blade works better if it is broken in two. These blades are easily broken off by clamping them in a vise that holds the entire length of the blade.

We have not found a cement that would not hold even though we have used water soluble glue. We have had most experience with Duco Household Cement and find it satisfactory. The blades are easily removed and the

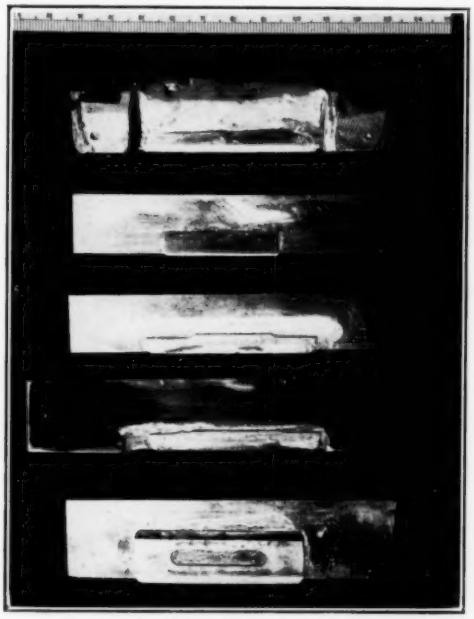

Fig. 1.—*1*, A regular microtome knife with the cemented blade projecting too much. *2*, A piece of sheet iron (gauge 16) with a hollow bevel. This sheet iron was hardly rigid enough when tested out on a block of dense leiomyoma about three-fourths inch square but worked on softer tissues. *3*, A piece of monel metal machined into the shape of a regular knife and an exaggerated hollow grinding. *4*, A piece of monel metal (same as No. 3) with a Schick blade cemented on the flat surface. This is our favorite set-up for small sections. *5*, A piece of babbit poured in a crude mold and dressed into shape with a rasp and emery paper. The babbit does not warm a frozen block or paraffin block as fast as steel or monel metal.

cement stripped off with an old discarded blade. No solvent is necessary but acetone may be used. Be sure to wipe off the oil or grease on new razor blades before gluing them.

A couple of horseshoe magnets are convenient for holding the blades together while the cement is setting.

The knife holders on some freezing microtomes are not adjustable and the resulting bevel is not always satisfactory. This may be compensated for in making the razor blade holder, or by the use of lugs, or by set screws properly added to the knife holder.

The illustration shows some makeshifts we have used with satisfactory results.

Mr. P. M. Schreck, Junior Medical Student at Baylor University School of Medicine at Dallas, Texas, assisted in this work.

TECHNIC FOR A BILE FISTULA IN THE RAT AND DEMONSTRATION OF THE INDISPENSABILITY OF THE BILE*

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MANY investigators have devised methods for studying bile. Each method has had an inherent difficulty which has led to other technics for excluding bile from the intestines. The dog has been the animal of choice in most bile fistula investigations recorded in the literature.

Of the many methods described for making biliary fistulas, one of the following procedures has been generally employed:

1. Suturing the gallbladder to the abdominal wall and opening it so that it drains to the exterior.
2. Suturing a cannula or rubber tube in the gallbladder.
3. Excising the common bile duct at the point of entrance to the duodenum, together with a piece of duodenal wall, and suturing the latter to the skin.
4. Placing a cannula in the common bile duct.

Bidder and Schmidt[1] in 1852 published their classical observations on the composition of fistula bile obtained from a simple external biliary fistula. Severe infections were produced, and the animals usually died within a very short period. The animals were able to lick themselves unless they were strapped all the time.

Dastre[2] collected bile from a fistula in a bag suspended from the neck of the animal. But, as Hooper and Whipple[6] point out with regard to similar findings in human beings with bile fistulas, there is no certainty that some bile did not reach the intestine, since autopsy findings are not available, and since in the light of our knowledge, new openings into the intestine may occur.

Pawlow transplanted to the skin a flap of duodenum containing the ampulla of Vater. Mann[7] described a method of transplanting a portion of the duodenum containing the common duct through an abdominal incision into the subcutaneous layers, the duct later being connected with the body surface.

Rous and McMaster[4] utilized a long tube attached to a cannula inserted into the common duct. The cystic duct was ligated and the bile collected in a sterile balloon.

Pearce and Eisenbrey[5] have suggested a different approach to the problem. In connection with studies on the relationship of the spleen to blood destruction, they found it necessary to develop some method by which bile might be

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collected quantitatively over long periods. They anastomosed the common duct to the right ureter after removing the right kidney. Although the original purpose of the technic was defeated because of the impossibility of separating chemical substances common both to urine and to bile, they published the method because of its suitability for other investigations.

Kapsinow, Engle and Harvey[8] described an intraabdominal biliary exclusion from the intestines through a cholecystnephrostomy. Since Pearce and Eisenbrey first introduced their technic for intraabdominal drainage, the biliary channels have been transplanted to many parts of the intestinal tract and urinary system.

The need for an exact means of bile collection is indicated by the fact that major problems in connection with the bile output which were first formulated nearly three generations ago are still unsettled.

The physiology of the bile and its relation to the various metabolic functions of the body has recently acquired renewed interest because of the work of Hooper and Whipple, Rous and McMaster, and others. In attempting to study the important question whether or not bile is essential to life, the physiologist has been confronted with the difficulty of finding some method which would not entail excessive inconvenience to the animal or to the investigator.

The ability of the white rat to adapt itself to almost any reasonable dietary regimen, and the economy of its maintenance, have resulted in its being the animal of choice in most physiologic laboratories. The rôle of bile in nutrition and metabolism has been almost universally accepted as important. Hence no such studies are complete without considering the bile factor. That the rat is without a gallbladder makes the interest of such studies even greater. For these reasons it is believed that there is need for a bile fistula technic which will meet the following requirements:

1. Bile must be completely eliminated from the animal so there remains no possibility of absorption or ingestion by mouth.
2. Collection at varying intervals must be carried out with ease.
3. The bile must be collected quantitatively and uncontaminated.
4. The animal should suffer a minimum of discomfort.

So far as is known, no technic for a bile fistula exists which meets all these requirements, except that described herein. The latter is the outcome of prolonged experimenting with internal and external reservoirs of rubber and glass, of many sizes and shapes, variously placed in the animal. It is believed that this technic meets most, if not all, of the requirements for a satisfactory bile fistula in the white rat.

The complete assembly of the collecting apparatus for carrying out the bile fistula operation is shown in Fig. 1. The glass bulb, *A*, serves as a bile reservoir. Its capacity may be varied according to the animal used. For animals weighing approximately 200 gm., a 6 c.c. bulb is employed. It has two nipples at the top, *B* and *E*, one placed vertically for withdrawal of bile, the other horizontally to admit bile. The nipple *B*, admits a 19 gauge needle and holds firmly in position a flexible rubber tube, *C*, 3 mm. inside diameter and approximately 4 cm. in

length. A short length of pressure tubing, *D*, fits tightly over *C*, to retain it on the outside of the animal. Attached to the nipple *E* is a piece of thin-walled, very flexible, 1 mm. rubber tubing, the length of which is fixed at operation. The cannula *G*, which is inserted into the bile duct, is made of 18 or 19 gauge stainless steel or silver hypodermic needle shaft tubing; its length is approximately 8 mm. There is a gentle taper from the center to the end which inserts in the bile duct. The duct is held in position by a fine silk ligature which fits into a narrow groove circumscribing the cannula about its center.

The operation is carried out with the animal under ether anesthesia. The anesthetic is efficiently administered through the use of the apparatus described by me,[9] substituting a mask for the tracheal cannula. Aseptic surgical technic is employed throughout all operative procedures.

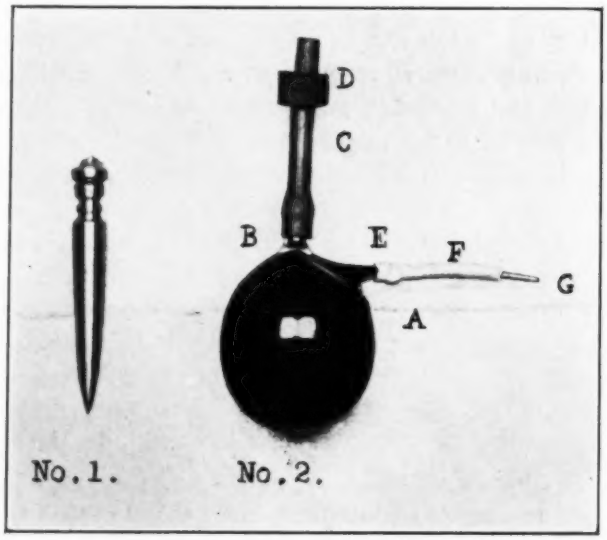

Fig. 1.—*1*, Special needle. *2*, Collecting apparatus (assembled).

The animal being properly prepared for operation, an incision is made in the right upper quadrant just lateral to the right rectus muscle, approximating in length the diameter of the bulb to be used. The duodenum usually comes into full view, and is pulled gently through the incision. The bile duct is seen coursing through the mesentery, and along the anterior wall of the duodenum for some distance. It is this section of the duct which is cannulated because it is firmly attached, its walls are strong, and the size of the lumen is greatest.

With a fine needle, a long silk ligature is placed beneath the duct close to its opening into the duodenum, and securely tied. The duct becomes filled with bile a few minutes following ligation. A second ligature is passed beneath the duct close to the first, avoiding certain vessels which are sometimes found coursing over the duct. With an iridectomy scissors, and using the loose ends of the first ligature as an anchor, the duct is hemisected; a blunt probe is introduced. The probe is withdrawn and inserted loosely into the untapered end of the cannula. Using the probe as a convenient handle, the cannula can be easily

inserted into the duct. The probe is removed from the cannula, and a second ligature made taut in the groove. Bile can now be observed to flow from the cannula. The entire duodenum is returned to the abdominal cavity, the ends of the ligatures remaining outside.

The rubber tubing *C* of the assembled bulb is slipped over the special needle shown in Fig. 1. The needle is passed through a point just lateral to the right sacrospinalis muscle about midway between the last rib and the innominate bone. The rubber tubing is next drawn to the dorsal surface, and the bulb is introduced into the abdomen. With forceps, the rubber tubing *F*, adjusted to a satisfactory length, can be securely slipped onto the cannula. The ends of the ligatures may here be utilized advantageously. The ends of the first ligature are used to secure the tubing on the cannula, and to retain both in line with the duct on the duodenum. The threads are cut close to their knots, and the duodenum is replaced in its normal position. The abdomen is closed in the usual manner, and the animal placed on its left side. The special needle is drawn through the short length of rubber tubing *D*, which is adjusted to a satisfactory position on tube *C*. Removal of the special needle completes the operation.

The exposed rubber parts are seldom destroyed by the animal. Should a rat be encountered which persists in chewing the tubing, a snugly fitting glass or metal tube may be placed over the rubber. This is seldom necessary if tame animals are used and kept in individual cages.

The tube *C* serves a dual purpose. It allows air to be expelled from the reservoir as bile flows in, and admits a blunt 7 cm. 19 gauge needle attached to a syringe of appropriate capacity for the withdrawal of bile. Merthiolate is used in the bulb as a preservative. The reservoir may be rapidly and completely emptied of bile with a minimum of discomfort to the animal.

In a series of experiments carried out to test the technic, female rats approximately five months of age were used. Females were given preference because of the additional information of the state of the animal afforded by following the estrous cycle. The animals occupied metabolism cages in a constant temperature room, and were fed a standard stock diet designated Diet I.* Food, water, urine, bile, and animal weights were measured twice daily, at 7 A.M. and 7 P.M.

Three groups of animals were designed to act as controls for the technic. Group I consisted of twenty animals, the bile ducts of which were ligated. Group II consisted of twenty animals which carried the entire collection apparatus; the bile duct was cannulated but a plug placed in the rubber tubing *C*, so that bile was unable to flow into the collecting bulb because of the air pressure encountered therein. Group III consisted of eleven animals which carried the collection apparatus, but the bile duct was not cannulated, a plug being placed in the tubing which ordinarily receives the cannula.

No differences were found between the reactions of Groups I and II. The length of life of these animals and their general downhill course closely ap-

*Diet I: Whole wheat, 67.5; casein, 15.0; whole milk powder, 10; sodium chloride, 1.0; calcium carbonate, 1.5; and milk fat, 5.0.

proximated each other. At autopsy, some fifteen to thirty-five days following operation, the animals of Groups I and II were found to have bile ducts greatly distended with bile, and livers presenting the usual destruction encountered in biliary obstruction.

The animals of Group III were practically unaffected by the operation. No change occurred in food or water consumption, or in urine output. These elements manifested the diurnal variation shown prior to operation. One hundred and twenty days postoperative, the animals had maintained or gained weight, and had continued to run normal cycles. A few of these animals were sacrificed after thirty days, and at autopsy were found to present little or no change attributable to the presence of the collecting apparatus.

A fourth group of animals constituted the experimental group, and upon them the complete technic was carried out so that the total output of bile drained into the reservoir. These animals recovered from the operation and appeared normal for from twelve to twenty-four hours. The next period was usually accompanied by a gradual decrease in activity, weakness, coma, and finally death. The food intake dropped to almost zero. The water intake was in most cases lowered, and the volume of urine considerably reduced. Some animals survived up to ninety hours. During the interval between operation and death, a tremendous loss of weight was noticed, far more than would be lost in a normal starving animal. Table I illustrates data which exemplify the results obtained in 6 animals over a period of forty-eight hours after the operation (with the exception of animal GH3640, which was observed for twenty-four hours).

TABLE I

THE DIURNAL VARIATIONS OF THE SECRETION OF BILE IN RATS

ANIMAL	WEIGHT AT OPERATION GM.	WEIGHT AT DEATH GM.	TOTAL LOSS IN WEIGHT GM.		BILE		SECRETION	
					7 A.M. C.C.	7 P.M. C.C.	TOTAL FOR 24 HOURS C.C.	C.C. PER KILO PER 24 HOURS
W3672	236	202	34	1st 24 hr.	4.4	6.8	11.2	50.4
				2nd 24 hr.	4.9	4.6	9.5	42.7
W3306	221	187	34	1st 24 hr.	4.8	6.5	11.3	55.3
				2nd 24 hr.	4.9	4.3	9.2	45.0
BH3436	211	178	33	1st 24 hr.	5.4	6.0	11.4	58.1
				2nd 24 hr.	4.9	4.9	9.8	49.9
W3318	240	200	40	1st 24 hr.	5.0	8.2	13.2	59.4
				2nd 24 hr.	5.1	7.3	12.4	55.8
BH3433	209	178	31	1st 24 hr.	3.9	5.7	9.6	48.9
				2nd 24 hr.	5.7	5.4	11.1	56.6
GH3640	249	220	29	1st 24 hr.	4.9	5.6	10.5	44.1

If at any time in the life of the animals in Group II the plugs were removed from tube *C*, allowing drainage of bile into the reservoir, they reacted exactly like the animals of the experimental group. If the plug were replaced before the animal lost appreciable quantities of bile, the animal would survive and continue in the same manner as the others of this group.

It is believed that the groups of animals acting as controls for the technic prove conclusively that the death of the animals in the experimental group was

not caused by the insults sustained during the operative procedure, nor by the presence of the collecting apparatus within the body cavity.

The lack of appetite, the tremendous loss of weight, and the rapid downhill course to death, again focus attention on the importance of bile in the life of the animal. It is believed that the fistula described will prove a valuable method of investigating further the rôle of bile in the life of the rat, an animal almost universally used for experimental purposes, whose biliary system differs from that of most vertebrates in that the gallbladder is entirely absent and without a homologue.

The writers wish to acknowledge with thanks the aid and guidance given to this work by Professor I. Maclaren Thompson, of the Anatomy Department of this university.

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THE USE OF PROTEINS FOR EMBEDDING SMALL TISSUES FOR SECTIONING BY THE FREEZING METHOD*

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THE summer of 1934 was so hot in this section of the country that paraffin cutting was quite a problem. As frozen technics are becoming quite generally adopted, the following method was developed instead of some better paraffin complex. This is not an impregnating method in its present form and cannot be used on tissues which do not hold together within themselves. However, nearly all the actual routine surgical bits of tissue can be blocked and sectioned by this method. It is economical and rapid.

The rationale of this method came as a result of speculations and reading about hyaline thrombi[1] and fixed colloids of various sorts in lymphatics, vessels, and open spaces. In routine tissues one often sees that formalin fixed proteins hold leucocytes and bits of tissue even though the cut section is passed through numerous solutions.

This method takes advantage of the fact that emulsoid colloids may be chosen which wet and stick to the tissues and which do not pass through membranes that are traversed by many coagulating substances. The advantage of using dialysis instead of adding the coagulant or fixative to the emulsoid is that the emulsoid has greater freedom to satisfy its particular water requirement and still not precipitate in disconnected clumps.

The emulsoid colloids which we have found the most satisfactory from the standpoints of good blocking properties, availability, and economy, are the blood and egg proteins. Different proteins require varying percentage concentrations, but whole egg and laked oxalated whole blood already contain enough protein.

Oxalated blood which is left over from clinical tests and which contains over 14 gm. of hemoglobin per 100 c.c. has been found convenient and satisfactory. Other modifications included in this paper may be used by those who cannot get blood or who feel that the blood used might be confused with blood states in the tissues. A small bit of saponin powder is most convenient for changing the suspensoid cellular hemoglobin into an emulsoid. When the bloods from various groups are pooled, it is possibly better to lake the cells of each blood before adding them to the pool. The oxalated blood may be allowed to stand until the red blood cells have settled, the plasma decanted into one container, the cells laked and added to a separate pool. Equal parts of each may then be mixed for uniformity.

Whole raw eggs make satisfactory tissue blocks. The yolk is broken and stirred into the albumen. Dr. J. M. Thuringer who recently had a brief intro-

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This method was presented to the Society of Medical Sciences of the Oklahoma University School of Medicine on October 18, 1934.

duction to my method has kindly informed me of the fact that egg methods of blocking tissues antedate the paraffin methods; that Neuman used egg albumen for embedding the vitreous body in 1862; and that many others have worked with egg proteins. Some of the old methods were similar to the one I developed in trying to imitate with oxalated blood that which may be seen in many inflammatory and pathologic tissues.

If one anticipates impregnating protein-blocked tissues with paraffin for very thin sections, our preliminary experiments indicate that the whole egg complex is possibly the best protein because the lipoids do not interfere with freezing methods or staining. With a *slow* method of paraffin impregnation, the extraction of the lipoids leaves a fine delicate reticulum into which the paraffin can permeate. Possibly better suspensoid substances can be developed and added to protein emulsions, especially the blood proteins. But the lipoids are already in such a highly disperse state in fresh eggs and are extracted readily by the dehydrating agents used in paraffin embedding that improvement seems difficult. A smooth emulsoid formalin fixed protein is rather resistant to paraffin impregnation.

Fig. 1.

Probably the most available commercial protein is egg white powder. This can be bought in candy factories cheaper than most other dehydrated proteins. Three grams of egg white powder in 10 c.c. of water, or one gram of egg white powder in 5 c.c. of blood serum, left over from serologic tests, has been found satisfactory.

We have not found any bacteriostatic agents up to the present time which can be added in sufficient quantity to the high protein content emulsions used. They alter the emulsoid state or interfere with staining. Prepared emulsoids can be kept in the refrigerator or be made fresh from the powdered protein in a few minutes. The search for a powdered protein was prompted by this difficulty in finding a bacteriostatic agent. We forgot to return to the refrigerator some laked oxalated blood and the odor of putrefaction which developed convinced us that those attempting this method should be warned and given some alternate procedure if they did not have good refrigeration. The age also of some emulsoids seems to affect their water binding power during fixation so that the blocks may not have as much volume as with fresh emulsions. But as long as small batches are made, they should not age sufficiently to affect their utility and accidental putrefaction then becomes a mere incident.

Several other commercial powdered proteins have been tried, such as: casco (casein) glue; monite (casein) glue; casec protein milk; dehydrated ox blood hemoglobin; dehydrated beef muscle; and dried buttermilk, sold for chicken feed. Each of these has some property that keeps it from being recommended.

Gelatin methods have been described[2] and are used to some extent. A high percentage of gelatin can be dissolved in blood serum and remain liquid at room temperature. But the expense is greater and the sections seem to shear

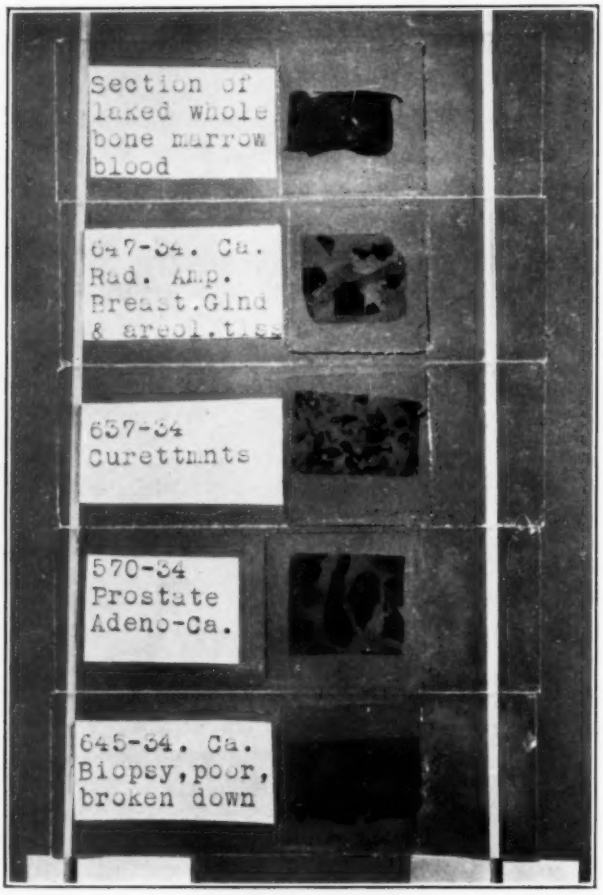

Fig. 2.

and crack. The use of plasma seems to improve the sections but the necessity of buffering the high calcium content of commercial gelatins keeps this from being recommended.

The dialyzing membrane is made by folding a piece of ordinary wrapping cellophane (not waterproof) measuring about 2 by 4 inches into the shape of a cubical box in the same manner that paper boxes are made for paraffin embedding. The cellophane is folded over a wooden block with square ends measuring about three-fourths of an inch square so that the ultimate block will be covered by a glass coverslip seven-eights of an inch square. If there are only a few very small bits of tissue, cut out a piece of heavy blotting paper the size

of the end of the wooden block and fold the cellophane over this. There is usually some wrinkling of the protein block. The paper keeps the bits of tissue in one plane. Thus one is assured of cutting and looking at all the bits of tissue submitted. The boxes should be as small as necessary to produce thin sections with the particular freezing microtome being used. The two projecting folds of cellophane are convenient when handling the box.

The coagulant used is formalin solutions from 20 to 40 per cent in strength. It does not interfere with freezing methods, and penetrates rapidly.

The steps in preparing tissue blocks may be summarized as follows:

1. Place the cellophane box in a small dish (we use low Stender dishes).
2. Place the bits of tissue in the box (previous fixation is optional).
3. Pour the emulsoid protein over the pieces and stir or orient them. Air bubbles of significant size should not be trapped at the interfaces.
4. Pour about 5 c.c. of 20 to 40 per cent formalin into the dish and around the cellophane box. The level of the formalin should be a little below the level of the emulsoid. It should be neutral or slightly alkaline so as to preserve the proteins about their isoelectric points. The dish may be covered to keep down the formaldehyde.
5. Allow from two to four hours for fixation. This depends on the amount of emulsoid used, the strength of formalin and the temperature of the room.
6. Peel off the cellophane. This is most conveniently done at the sink, allowing running water to wash off the strong formalin.
7. Trim the block and proceed as with any formalin fixed tissue.

The satisfactory reports that have come in to me from workers to whom the above outline has been submitted confirm our experience and seem to justify publication at this stage.

Dr. H. Nauheim (Pathologist at Morningside Hospital, Tulsa, Oklahoma) informs me he has blocked bits of tissue removed by the needle puncture biopsy method with good success.

Other objects besides tissue such as parasites and fecal concentrates containing parasite ova may be blocked and sectioned for mounting.

Those who happen to use fresh oxalated blood will discover intact leucocytes in the blocking media. This observation led to studies on bone marrow blood and this preliminary report must be included at this time. Dr. E. E. Osgood demonstrated, at the American Medical Association Convention at Cleveland, Ohio (1934), a method of drawing off bone marrow from the sternum with a needle, oxalating it and making smears in the same manner that smears are made from oxalated whole blood. The oxalated bone marrow blood may be laked and fixed by this method. In thin sections the cells may then be studied in their spherical shapes by the usual histologic methods. If the patient is anemic, the bone marrow blood proteins may be enriched with some stock laked red cell hemoglobin emulsion. We have had no leucemia cases to try this method on, but anticipate no contraindication.

SUMMARY

A simple method is described in which emulsoid proteins are used for blocking routine surgical bits of tissue.

Special consideration is given to freezing and routine staining technics.

The principle of dialysis is utilized and the reasons for its use are given.

Cellophane is used for the first time so far as we can determine. It simplifies the application of dialysis.

The use of laked whole oxalated blood is used for the first time so far as we can determine.

The possibility of studying leucocytes and bone marrow cells in the blood and in their spherical shapes is suggested.

Mr. P. M. Schreck, junior medical student at Baylor University School of Medicine, Dallas, Texas, kindly assisted with this problem during his summer vacation.

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A SUSPENSION DAMPED FOR LOW FREQUENCY VIBRATIONS*

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IN CONNECTION with some work in a third floor laboratory, it was found impossible to use a sensitive galvanometer because of the vibrations continually occurring. We are deeply indebted to Dr. L. S. Jacobsen, of the Mechanical Engineering Department, for suggesting methods by which these difficulties could be surmounted. The most difficult part of the situation was the relatively low frequency of the vibrations, which were eighty-five per minute and were due to walking. Since such a situation very commonly arises, our experience in its solution may be of sufficient interest to justify the detail given in this note.

With the intention of having the galvanometer, source of light, and other parts of the apparatus, all suspended, a seventy-five pound slab of marble, 57 by 83 by 4 cm., was hung from the ceiling by four straps cut from automobile tire inner tubes. The part of the room where the apparatus was to be set up had a slight slant on the ceiling, and therefore the bands had to be of different lengths. The relation between the period and the strength or static deflection is given by the equation:

$$N = \sqrt{188 \quad \frac{1}{\triangle \text{ St}}}$$

where N = cycles per minute
$\triangle$ St = static deflection in inches

All rubber bands were brought to the same period by loading equally and then trimming down to a width that gave equal frequency.

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The accompanying figure shows the relation of static deflection to vibrations per minute for 4.7 and 10 cm. wide rubber bands. It appears that rubber does not follow the above equation perfectly for the wider band requires greater static deflection to attain the same period.

Escape from vibration is best accomplished by making the period of the suspended mass twice the period of the vibrations of the building. Since it was desired to escape vibrations of a frequency of eighty or more, the curve for the

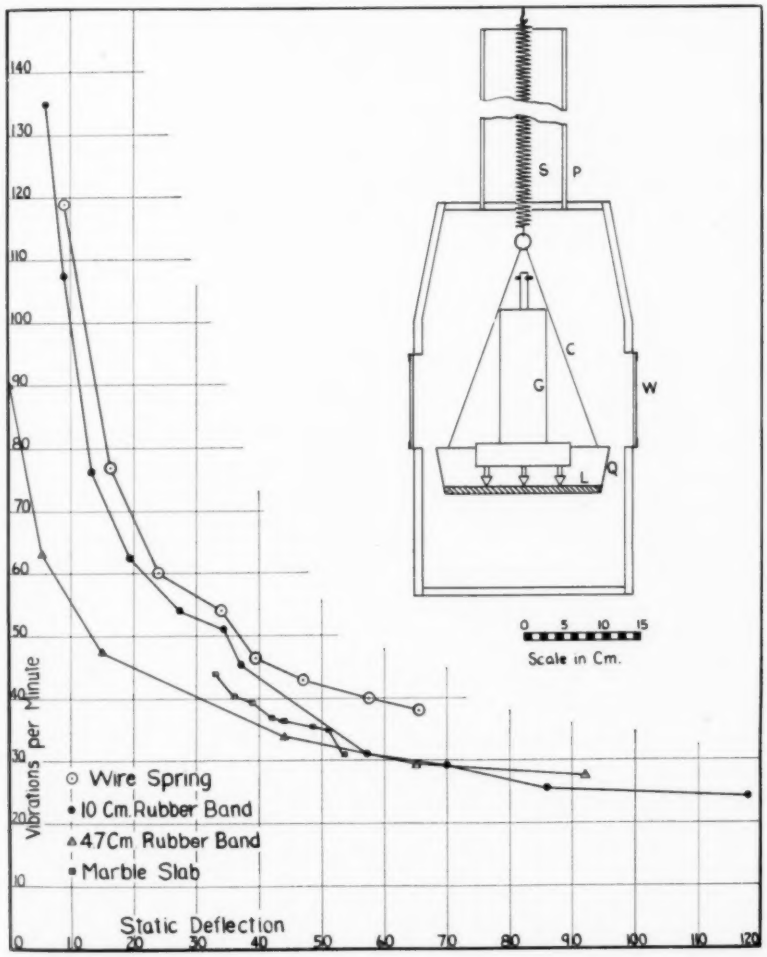

Fig. 1.

period of the suspended marble slab shows the accomplishment of this aim. It was found, however, that the frame of the building changed its position slightly with the wind and this made it necessary to adopt a one point suspension method.

Rubber has the advantage over springs in that it has no resonance, but springs follow the above equation more perfectly. However, for the single suspension method a spring of No. 12 coppered Bessimer steel with 300 turns of $\frac{3}{8}$ inch radius was used. The static deflection of the spring required to give the proper period is shown in the figure. When thus stretched the spring was 203 cm. long.

To support the galvanometer (G), the handle was removed from a 20 cm. cast-iron frying pan (Q) and three holes were bored into the sides at points 120° apart, from which were run chains (C) to the end of the spring (S). The weight required to give the correct period was 11.15 kg. and the weight of the galvanometer was 1.85 kg. Melted lead (L) was poured into the pan until the pan and lead were brought to the required weight, i.e., 8.30 kg. The lead formed a flat surface on which it was easy to level the galvanometer. To protect the spring from air currents, we put a galvanized sheet-iron pipe (P) around it and fastened that to a square box surrounding the pan and galvanometer. Two sides (W) of this lower box were of glass which could be slipped in and out making the instrument easy to handle. The galvanometer and the weighted pan gave approximately the desired period, and therefore were not appreciably affected by the vibrations of the building. Thus by application of the equation relating static deflection to period, vibrations of low frequency can be avoided. In our case we succeeded in obtaining a suspension of sufficient steadiness for proper functioning of a quadrant electrometer.

A NEW RAT FEEDING DEVICE*

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MOST rat feeds are in the form of powders because of the nature of the ingredients used in nutritional studies, but this form is inconvenient in several respects. In the first place, the powder is blown throughout the animal breeding room. Second, it is impossible to determine how much of the food is wasted by mixing with the sawdust bedding. Third, no containers are effective in preventing contamination of the food.

There are now on the market several adequate rat diets in the form of compressed cylindrical bodies about 5 mm. in diameter and 10 to 20 mm. long. These are quite uniform in diameter as though forced out from an orifice in the process of manufacture. The constituents of the pellets are such as to provide a complete diet. Moreover, the hardness of this type of food provides a grinding surface for the rats which is completely absent in the powder form diets. Rats which are fed the latter compensate by grinding their teeth on available parts of their cages and the copper tubes of their water bottles with a surprising amount of damage resulting to both. The new pellets have a more natural effect on the rats' teeth and eliminate much damage to equipment.

In Fig. 1 is shown an apparatus which enables the rat to obtain compressed food pellets at will, and at the same time prevents contamination and loss of food. The device consists of a brass tube an inch and a half in diameter and seven inches long, suspended by a hook in the upper end, and the lower end somewhat flattened and provided with teeth. A stiff wire is suspended loosely down

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the full length of the tube and projects beyond the orifice to end in a loop. Such tubes may be detached from the cage, filled with the pellets, and resuspended in a few moments. The jaws of the tube are so arranged that in the process of withdrawing the pellets the rat agitates the tube to some extent and this, together with the loop of wire, prevents the pellets from clogging and causes them to continue to fall to the lower end of the tube. The rat seems to enjoy this type of feeding. He immediately seizes the pellets in his jaws and pressing with his forefeet on the tube exerts considerable strength, and thus succeeds in pulling

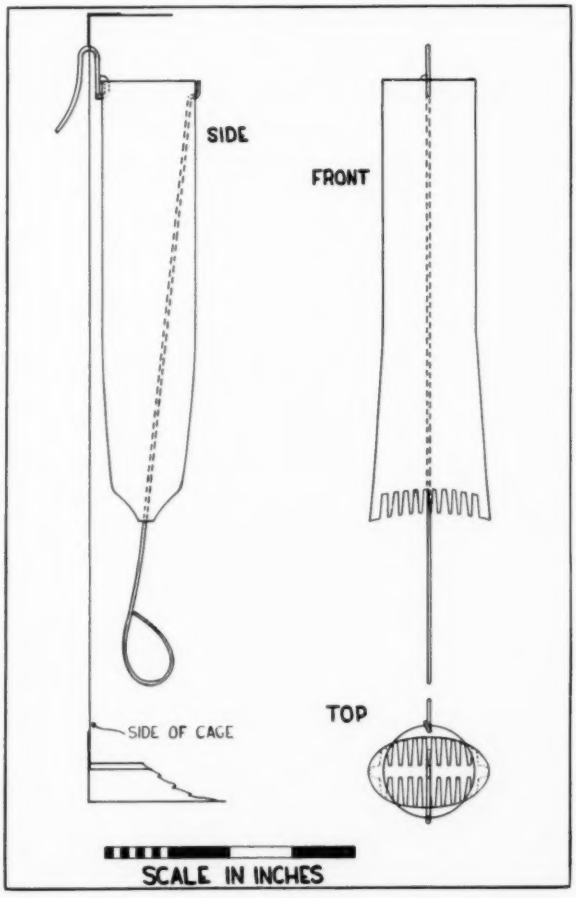

Fig. 1.

the pellets out in spite of the tight set of the jaws. Usually when the tubes are refilled, several rats will attempt to obtain pellets at the same time, crowding each other away from the tube. This jostling seldom leads to a fight but is all in good nature. When a pellet is obtained, the rat retires to the other side of the cage and remains there sitting on his haunches very much like a squirrel with a nut. Occasionally rats develop the habit of emptying the feeding tube completely. They store the food in one corner of the cage, but in all cases, the food thus stored during the course of the day is eaten. A tube of the size indicated contains food for six rats for a day.

A METHOD FOR THE DETERMINATION OF CREATINE IN URINE*

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BUFFALO, N. Y.

DURING a study of the metabolism of creatine in cases of muscular dystrophy, we compared several methods for the determination of creatine in urine, and with each of them obtained values which were shown to be incorrect. With deeply pigmented urines, the values were often two, and occasionally three times too large. An amount of extra chromogenic material was formed by the action of acid and heat upon some constituent of the urine, and the analysts did not distinguish the color which was due to creatinine from that which was produced by the other chromogenic material. Persons whose eyes were more sensitive to shades of red, and who could match the dissimilar shades in the colorimeter, obtained moderately accurate values for the creatine. Similar difficulty was reported by McCrudden and Sargent,[1, 2] and has been experienced by others.

It was found also, that when extra color was absent, the conversion of creatine into creatinine was often incomplete. This inadequacy has been noted by Hahn and Barkhan,[3] who determined creatine of urine by heating the solutions only to 65°, and allowing eighteen to twenty-four hours for the conversion.

We have tested the conversion of urinary creatine into creatinine by concentrated sulphuric acid. Under certain conditions, the conversion was completed in two hours, and a negligible amount of extraneous color was developed. A simple procedure was therefore devised which enabled accurate analyses to be obtained for creatine in urine. Diabetic urines could not be used.

PROCEDURE

Pipette 1 c.c. of urine into a 100 c.c. volumetric flask. Add, from a pipette, 1 c.c. of concentrated sulphuric acid, mixing after each drop. One or two minutes are required for this addition. Allow the flask to stand at room temperature for two hours. When 2 c.c. of urine are used for the analysis, the flask should stand for five hours.

Into a similar 100 c.c. flask pipette 1 c.c. of standard creatinine solution (1 c.c. = 1 mg. of creatinine), and then add to it 1 c.c. of concentrated sulphuric acid.

To each flask add 20 c.c. of a saturated solution of picric acid (purified if necessary), and then 10 per cent sodium hydroxide so that an excess of 1.5 c.c. over that needed to neutralize the sulphuric acid is present. In our analyses 14 c.c. were used. Precipitation of picric acid or its salt may be ignored. After ten minutes, dilute each solution to the mark, mix, and compare in the colorimeter. The calculations are made as usual.

In Table I, figures to the nearest even percentage are given to illustrate the rate of conversion of creatine into creatinine under the conditions described. One cubic centimeter portions of creatine in distilled water were permitted to stand

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with 1 c.c. portions of concentrated sulphuric acid for periods varying from one minute to seventeen hours. Duplicate analyses were always made. The conversion was complete in two hours, and further increase of time did not alter the value.

TABLE I

CONVERSION OF CREATINE TO CREATININE BY CONCENTRATED SULPHURIC ACID

SAMPLE	CONTENTS	TIME	CREATININE RECOVERED	RECOVERED
			MG.	%
1	1 c.c. creatine solution*	1 min.	0.00	0
2	1 c.c. creatine solution*	10 min.	0.10	10
3	1 c.c. creatine solution*	1 hour	0.70	70
4	1 c.c. creatine solution*	2 hours	1.00	100
5	1 c.c. creatine solution*	5 hours	1.00	100
6	1 c.c. creatine solution*	8 hours	0.96	96
7	1 c.c. creatine solution*	17 hours	1.00	100

*1 c.c. of creatine solution equals 1 mg. of creatinine.

In Table II are shown values for creatine in urine which contained much pigment. The small amount of color that developed from the action of concentrated sulphuric acid on the urine pigments did not affect the values for creatine. As a further test of the color, 1 c.c. portions of urine were allowed to stand with sulphuric acid for periods ranging up to eight hours. Sodium hydroxide was then added, and the solutions were diluted to 100 c.c. and compared in the colorimeter with 1 c.c. portions of urine which were made alkaline and were diluted directly to the same volume. No difference in the fields could be observed.

TABLE II*

ANALYSIS OF A HIGHLY PIGMENTED URINE FOR CREATINE BY USE OF SULPHURIC ACID

SAMPLE	CONTENTS	TIME	TOTAL CREATININE
			MG.
1	1 c.c. urine	1 min.	2.09
2	1 c.c. urine	3 min.	2.02
3	1 c.c. urine	12 min.	2.07
4	1 c.c. urine	1 hour	1.96
5	1 c.c. urine	2 hours	2.11
6	1 c.c. urine	3 hours	2.11
7	1 c.c. urine	17 hours	2.11

*Analysis of the urine for "Preformed Creatinine" by the Folin method gave values ranging from 2.08 to 2.11 mg. per c.c. Analyses for creatine by other procedures gave deep red colors which corresponded to values of from 5 to 10 mg. of creatine per c.c.

In Table III, figures are given to show the influence of an excess of 10 per cent sodium hydroxide on the development of the color. One cubic centimeter samples of the standard creatine solution (1 c.c. = 1 mg. of creatinine) were analyzed by the sulphuric acid method. The amount of 10 per cent sodium hydroxide required to neutralize the acid and to develop the color was 14 c.c. Larger amounts of sodium hydroxide were added to the different samples and each was compared in the colorimeter with a specimen that had received the 14 c.c. of sodium hydroxide. The analyses show that 2 c.c. of sodium hydroxide over the amount needed did not alter appreciably the color which was developed, and that larger amounts decreased the color.

TABLE III

THE INFLUENCE OF EXCESS OF SODIUM HYDROXIDE ON THE COLOR DEVELOPMENT

SAMPLE	CREATINE CONTENT	10% SODIUM HYDROXIDE ADDED	CREATININE DETERMINED	RECOVERY
		C.C.	MG.	%
1	Equivalent to 1 mg. creatinine	14.0	1.00	100
2	Equivalent to 1 mg. creatinine	14.2	1.00	100
3	Equivalent to 1 mg. creatinine	14.5	1.02	102
4	Equivalent to 1 mg. creatinine	15.0	1.00	100
5	Equivalent to 1 mg. creatinine	15.5	0.98	98
6	Equivalent to 1 mg. creatinine	16.0	0.98	98
7	Equivalent to 1 mg. creatinine	16.5	0.90	90
8	Equivalent to 1 mg. creatinine	17.0	0.90	90
9	Equivalent to 1 mg. creatinine	18.0	0.87	87
10	Equivalent to 1 mg. creatinine	20.0	0.78	78

TABLE IV

RECOVERY OF CREATINE ADDED TO URINE

SAMPLE	SOURCE	PREFORMED CREATININE	TOTAL CREATININE	CREATINE ADDED	CREATINE DETERMINED	
		MG./C.C.	MG./C.C.	MG.	MG./C.C.	%
1	Adult male	2.10	2.57	1.00	3.53	96.0
2	Adult male	1.95	1.93	1.00	2.91	98.0
3	Adult male	2.74	2.82	1.00	3.87	105.0
4	Child	0.78	1.00	1.00	2.00	100.0
5	Adult male	2.11	2.06	1.00	3.01	95.0
6	Adult female	2.72	3.45	1.00	4.46	101.0
7	Child	0.72	1.25	1.15	2.45	104.0
8	Adult male	1.06	1.43	1.15	2.61	102.6

Moderate variations in the temperature (from 0 to 25°) did not influence the values obtained.

In Table IV are given values which were obtained by analysis of several urines with the sulphuric acid procedure. Known amounts of creatine were added to the urines and attempts were made to determine the same. In each case the recovery was quantitative within the limit of accuracy of the colorimeter. The method has been used by us over a period of several months, and has been repeatedly tested as to its accuracy.

CONCLUSIONS

A simple method for the analysis of creatine of urine by use of sulphuric acid is described. Extraneous red color, such as occurs in certain other procedures, is not formed, and creatine which has been added to urine may be accounted for quantitatively.

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ALTERING THE VIM-SCHEFTEL COLORIMETER FOR GENERAL USE*

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FOR the small laboratory the expense of a colorimeter is a considerable item. There is on the market a relatively inexpensive instrument, known as the Vim-Scheftel colorimeter, which is of proved reliability and serviceability. As it is at present marketed, however, it is supplied with a syringe calibrated in arbitrary units, so that it is necessary to refer a reading to tables prepared by the manufacturers. At present the colorimeter has been standardized for only a few of the more common procedures such as the phenolsulphonephthalein kidney function test, nonprotein nitrogen of the blood, blood urea, and blood sugar. As it stands the colorimeter cannot be used for other work such as blood creatinine, blood phosphorus, icterus index, bilirubin liver function test, and so on, without tedious calibration.

However, the instrument can readily be changed into a general colorimeter without additional expense. This can be accomplished simply by using an ordinary tuberculin syringe in place of the one which is provided with the instrument. It is then usable with the ordinary colorimetric formulas.

Two cubic centimeters of the standard solution is pipetted into the tube for the standard, and 1, 2, or 3 c.c. of the unknown color solution is pipetted into the unknown tube with the tuberculin syringe attached. Enough must be used so that the color is more intense than the standard. The solution is then drawn back into the tuberculin syringe until the colors of the standard and the unknown are matched in the eyepiece. The reading of the unknown will then be the amount originally pipetted into the unknown tube minus the reading on the tuberculin syringe, viz. 3.0 c.c. – 0.35 = 2.65 c.c. the reading of the unknown. The usual formula can then be followed as in the blood creatinine determination by the method of Folin.

$$\frac{\text{Reading of Standard}}{\text{Reading of Unknown}} \times 1.5 = \text{mg. creatinine per 100 c.c. blood which would be } \frac{2.00}{2.65} \times 1.5 = \text{mg. creatinine per 100 c.c. blood}$$

The instrument with the tuberculin syringe used in this way has been checked against a large prism colorimeter and has been found to be accurate within the usual limits of error.

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A METHOD OF SPUTUM DIGESTION PRELIMINARY TO CULTURE FOR TUBERCLE BACILLI*

W. L. McNamara, M.D., and E. F. Ducey, M.D., West Los Angeles, Calif.

DIGESTION is a vital step preliminary to the culture of tubercle bacilli from biologic specimens such as sputum, urine, feces, and exudates. This must accomplish the killing of extraneous organisms, as well as the freeing of the tubercle bacilli from caseous masses. While digestion may be done in several different ways the use of either acids or alkalies is the method most generally employed at present. Pinner recommends equal parts of sputum and 3 per cent hydrochloric acid for a period of thirty minutes. Petroff uses equal parts of sputum and 3 per cent sodium hydroxide from thirty to forty minutes. We have found that when the period of digestion with acids or alkalies is extended for four to six hours many of the tubercle bacilli are killed. This suggests the possibility that a certain number of organisms may be killed within a minimal period of time, a factor of importance in specimens in which the total number is small.

We employ the following method for digestion, and have found that it is entirely safe, having recovered viable organisms often after seventy-two hours of digestion in the incubator; the sputum is completely liquefied and freed from small caseous masses which may harbor spores.

Method of Digestion: 10 c.c. of sputum is placed in a 50 c.c. centrifuge tube (sterile), 20 c.c. of 1 per cent hydrochloric acid and 100 mg. of pepsin (Stearns 1-12,000) are added. The tube is closed with a rubber stopper which is secured with rubber bands or adhesive tape to prevent leakage. The tube is placed in a sputum shaker or Kahn shaker for fifteen minutes and is then placed in the incubator for twelve hours. It is then neutralized to litmus with normal sodium hydroxide and centrifugated for one hour.

Tubes and plates are inoculated with material aspirated from the bottom of the tube with a sterile capillary pipette.

With organisms recovered by this method of digestion we have been able to reproduce tuberculous lesions in guinea pigs which were verified by histologic examination, and have obtained typical growths on culture media. We feel that the greater amount of time consumed by this method is more than compensated by the fact that the danger of killing the tubercle bacilli is reduced to a minimum.

*From the Clinical Laboratory, U. S. Veterans Administration Facility.
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A MASS CULTURE APPARATUS FOR SECURING BACTERIAL CELLS FOR ANALYSIS*

ROBERT A. GREENE, PH.D., TUCSON, ARIZ.

A RECENT study[2] of the chemical composition of the genus Azotobacter has necessitated some means of securing large amounts of bacterial cells. Since the moisture content of these bacteria is approximately 85 to 90 per cent, a considerable quantity of the organisms must be "harvested" in order to secure sufficient material for any extensive chemical analysis.

A mass culture apparatus, as described by Vaughan[4] was not available, and neither were monel metal culture dishes of the type used by Huston, Huddleson and Hershey.[3] A very large number of culture dishes of the usual size were required to give any appreciable quantity of cells, so, after considering the various methods which have been compiled by Buchanan and Fulmer,[1] the following apparatus was used:

Pyrex glass trays, whose dimensions were 18 by 12 by 2½ inches were used as the culture dish. At first, enameled steel instrument trays were used as covers. Later copper covers, which were constructed locally, were used. These were more satisfactory, since they could be snugly fitted to the dish, and also because they were more resistant to hot air sterilization.

The cost of a dish and cover (in quantity lots) is approximately six dollars. Each dish had about 216 square inches of surface, which is readily accessible when the bacterial growth is to be harvested. Approximately 22 ordinary sized culture dishes (100 by 10 mm.) are required to give this area. At the present price of culture dishes (approximately 24 cents for routine grade, 36 cents for a good grade of resistance glass, and 50 cents for Pyrex glass), the cost of dishes to give the same surface as one large Pyrex dish would be slightly less expensive in the routine grade, but would be more expensive if dishes of resistance or Pyrex glass were used.

The mass culture dishes are sterilized in the hot air sterilizer in the usual manner; the media are prepared in large flasks, sterilized in the autoclave, allowed to cool to about 45° C., and then poured into the dishes. When the media has solidified, the dishes are inoculated by placing a small quantity of the bacterial suspension on the surface of the agar. This may be spread over the surface by tipping the plates, but preferably by smearing with a bent, flat-tipped glass rod, or a sterile swab.

At the end of the incubation period, the growth is scraped from the surface of the media, taking care not to remove any of the agar. A glass slide or a celluloid spatula[3] may be used. The material may be dried according to any

*From the Department of Agricultural Chemistry, University of Arizona.
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standard method, but I have found that desiccation in anhydrous acetone, as suggested by Wilkerson and Gortner[5] is very satisfactory.

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A VASELINE DISPENSER FOR HANGING DROP PREPARATIONS*

ROBERT A. GREENE, PH.D., TUCSON, ARIZ.

NUMEROUS methods have been used to dispense vaseline in the preparation of hanging drop mounts, but many of these are not especially satisfactory, particularly for use in crowded student laboratories. The following method has been used in the Soil Microbiology Laboratory at the University of Arizona: an empty tube, which has a long, slender "nozzle" is packed with vaseline, and is used to dispense the vaseline for the hanging drop preparations. The types of containers used for nasal ointments are satisfactory, and may be secured from druggist's supply companies. The use of such a dispenser in a student laboratory has resulted in neater hanging drop preparations.

*From the Department of Agricultural Chemistry, University of Arizona.
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A SPECIAL CAMERA FOR ROUTINE PHOTOMICROGRAPHY*

J. A. Reyniers, M.S., Notre Dame, Ind.

FROM time to time, pocket or box cameras have been suggested for use in photomicrography. Most of these are makeshift devices intended for emergency use or as a substitute for expensive apparatus.

There is in photomicrography, as distinct a need for a small camera which can be manipulated quickly and which will allow inexpensive exposures to be

Fig. 1.

made, as there is need for a plate camera. Obviously, a film camera with a small-sized picture frame is not as satisfactory in some instances as a plate camera but on the other hand the inconvenience and expense of using a plate camera in routine work are factors which must be considered. It goes without comment that exact pictures can be obtained with small-sized-film negatives which can then be enlarged.

*From the Laboratory of Bacteriology, University of Notre Dame.
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The camera described in this paper has been designed for a definite purpose, i.e., routine photography. It has been in use for over two years in this capacity and has been most satisfactory. It is attached directly to the microscope in such a manner that a single motion will swing it aside so that there is no interference with the normal use of the microscope. The operator can see the exact field that he wishes to photograph and can focus the camera with precision. The entire apparatus is mechanically exact and very light.

The apparatus, Fig. 1, consists of a small film box built to use regular pocket size film and capable of taking 16 exposures on a standard 8 frame roll. The film is held perfectly flat against a spring. The box is mounted on an arm which is made from thin metal and pivoted to another arm which can be attached directly to the tube of the microscope by a special self-centering collar. This arrangement permits the upper arm bearing the camera to be swung from side to side. A viewing device is mounted next to the camera. A strip of film is used to screen the image. The diameter of this viewing device is such that it will cover an entire field as projected by a 10X ocular. Film is used in place of the usual ground glass because of its extremely fine-grained surface. The whole outfit is rigidly constructed. To be sure that the viewing device and the film are on exactly the same plane, the upper arm is guided by a groove in the lower arm. Because of the lightness of the camera and its particular construction, it does not bear directly on the tube of the microscope, consequently it does not interfere with the focus.

The camera remains attached to the microscope and is swung to one side when not in use. When a field is photographically desirable the image finder is brought into place and is checked by a click stop; the image is focused sharply and the light adjusted. The camera is moved into position and is held by a similar stop. The image is then photographed after which the camera is again pushed out of the way and observations are resumed. So easy is this motion that an operator familiar with photographic conditions and using a fast film can scan and photograph living forms with reasonable success.

This device has a wide range of usefulness and is especially valuable in the hospital or pathologic laboratory as well as in routine bacteriology or cryptogamic botany.

AN ELECTRODIALYSIS APPARATUS FOR USE WITH ALTERNATING CURRENT*

E. J. Czarnetzky, Ph.D., San Francisco, Calif.

ALTHOUGH there are several types of apparatus described in the literature, they are as a rule expensive and not entirely satisfactory. The present set-up is inexpensive and the parts are readily replaceable. It has the additional advantage of ease in changing the membranes without disturbing the solution to be dialyzed.

The electrodes are carbon or graphite rods which are placed close together to facilitate rapid ionic transfer, leaving room for a large volume of solution. Where metal salts, such as silver, are to be dialyzed, gold electrodes can be used on the negative side so that the metal is plated directly on the electrode, and thus furnish a measure of the extent of dialysis by determining the difference in the weight of the electrodes before and after plating. Various grades of DuPont cellophane can be used for the membranes. The cellophane is attached to the electrode cylinder by means of DeKhotinsky cement.

Fig. 1 shows the details of the apparatus. The ammeter is connected in shunt and can be connected by throwing the switches. If two 250 watt lamps are used in the cleats *I* and *J*, there will be approximately five amperes traveling through the system, provided it is not held back by the solution being dialyzed. The lamps also serve as indicators for the continuity of the system. Usually about 50 volts are required, and five amperes to start. As the concentration of electrolytes in the solution *Q* becomes less and less, the amperage will fall. When it has reached a figure close to zero, the dialysis is complete. The amperage is controlled somewhat by the rectifier. The latter consists of an electrode of tantalum metal of large surface and one of lead in a solution of sulphuric acid. The sulphuric acid coats the tantalum with a unimolecular layer of oxide, which is unipolar, and therefore only carries current in one direction. (Tantalum can be bought for about $1.50 per gram from the Fansteel Company, North Chicago, Ill.) It is necessary to use insulators as indicated, so that the current can be forced through the solution *Q*.

When the substance to be drawn out of the mixture is a simple salt such as sodium chloride, the amount of salt can be determined by titrating the solution in container *N*. This gives equivalents of base, and thus equivalents of monovalent salts. Cooling is provided by the flow of dialyzing water through *O* and *P*.

The flow of water from containers *F* and *G* should be slow so as to leave enough electrolyte in the system to carry the current. The dialyzing chambers

*From the Research Laboratories of the Mount Zion Hospital.
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O and *P* should have their side arms near the top of container *R* so that extra water is not forced into the solution *Q* by hydrostatic pressure. Where the amount of solution is small, use a smaller vessel, *R*.

Where ampholytes are used there will be some interference by reason of the Donnan effect, but this is overcome finally by the force of the current, so

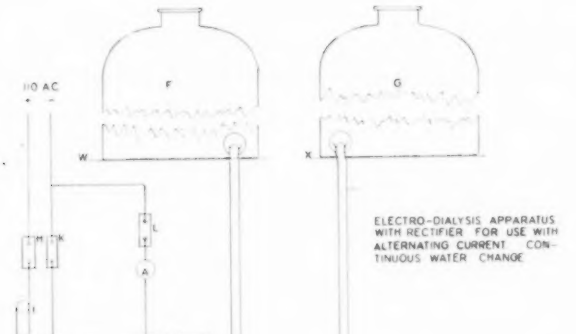

Fig. 1.—*A*, Ammeter in shunt. *B*, Tantalum rectifier. *C*, Lead electrode. *D*, Tantalum electrode with large surface. *E*, Five per cent sulphuric acid electrode solution which oxidizes the tantalum electrode with a unimolecular coat of oxide, which is unipolar and does not emit electrons. *F*, and *G*, Large glass jars containing distilled water which act as reservoirs with outlets leading to the electrode cylinders. *H*, Switch to start and stop current. *I* and *J*, Two pony cleats for holding lamps for the purpose of furnishing resistance to the current. They prevent fuses from blowing, and also are useful in adjusting the amperage. When they are lit it is a sign that current is flowing through the solution being dialyzed. *K*, and *L*, Switches for connecting the ammeter (*A*) in the line. *M* and *N*, Reservoirs for catching the spill over waste solutions which come from the dialyzing chambers. *M* contains waste acid as it is the positive pole. Conversely. *N* contains alkali as it is the negative pole. *O* and *P*, Electrode cylinders made of Pyrex glass. *Q*, The solution to be dialyzed. *R*, Glass jar for holding the solution to be dialyzed. *S* and *T*, Side arms for conducting the waste fluids to the reservoirs. *U*, Glass or rubber plate for insulation. *W* and *X*, Glass or rubber plates for insulation. *Y* and *Z*, Cellophane membranes covering the open ends of the dialyzing cylinders.

that little if any electrolyte remains in the solution. When dialyzing haptenes and substances of that nature attached to proteins, use a smaller current so that the compounds are not pulled apart.

Caution: When making the rectifier, leave an air hole in the top so that H_2 and O_2 formed by electrolysis can escape.

A NOTE ON THE DETERMINATION OF UREA IN BLOOD BY THE FOLIN DISTILLATION METHOD*

Determination of Urea by Steam Distillation

F. H. L. Taylor, and Margaret A. Adams, Boston, Mass.

THE steam distillation method described below was developed for the single purpose of eliminating the close supervision required by the present Folin-Wu[1] urea method and the modification of Lewis[2] or the newer method of Peskett.[4] To the many workers in the biochemical field, using the distillation method for urea, it is presented as a modification which has been used with success in many determinations of urea during the past two years and which eliminates the technical difficulties of backsucking of the distillate and too rapid evolution of ammonia. These two difficulties are often extremely difficult to eliminate in the older Folin method even with the closest attention. The modification includes the precaution of cooling the distillate (presented by Lewis) which has been used routinely in this laboratory as a useful precaution for some years.†

Apparatus.—A diagram of the apparatus is shown in Fig. 1. A = Erlenmeyer flask containing distilled water which has been boiled for about ten minutes. A two-hole rubber stopper in flask *A* contains: *B*, a short straight piece of glass tubing at the top of which is a 2-inch piece of rubber tubing and a pinch clamp; and *C*, which is a glass delivery tube of about 7 mm. bore leading from the flask *A* to the bottom of the distilling tube *D*. *C* may be one piece of glass tubing with angles in two places, or it may be composed of two glass bends connected by a short piece of rubber tubing, provided the ends of the two pieces of glass tubing are in contact with each other.

D is a Pyrex ignition tube containing the material in which the urea N is to be determined. By means of a two-hole rubber stopper in the neck of *D*, a delivery tube leads into the receiving tube *F*.

E is best prepared from a 5 or 10 c.c. volumetric pipette, since the bulb of the pipette still further decreases the possibility of sucking back from *F* into *D*. The pointed end of the pipette may be removed to make the delivery tube the desired length, but it is advisable to fire-polish the delivery end to slightly decrease the bore of the tube. A slight bend is made in the bottom of *E* so that the end of *E* will always be beneath the surface of the liquid in the receiving tube *F*.

*From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital and the Department of Medicine, Harvard Medical School.

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†The reagents and general procedure used are taken directly from Folin's outline of procedure.[3]

F is a test tube graduated at 15 and 25 c.c. During the distillation, *F* should be immersed in a beaker *G* containing ice water or water kept cool by constant flow.

Procedure.—Transfer 5 c.c. of tungstic acid blood filtrate to a Pyrex ignition tube (*D*). This tube must be rinsed with HNO_3 and water if it has previously contained Nessler's solution. Add 2 drops of buffer mixture and 1 c.c. of urease solution or a piece of urease paper. Stopper, and immerse the tube in warm water (37.5° C.) for fifteen minutes. Transfer 2 c.c. of 0.05 N-HCl to a test tube graduated at 15 and 25 c.c. (receiving tube *F*). Attach the receiving tube to the distilling apparatus in such a way that the end of the delivery tube *E* is under the surface of the liquid in receiving tube *F*, add to tube *D* a glass bead, 2 or 3 drops of paraffin oil, and 2 c.c. of saturated borax solution, and attach the tube so that the end of *C* is under the surface of the liquid in *D*. The tube *D*

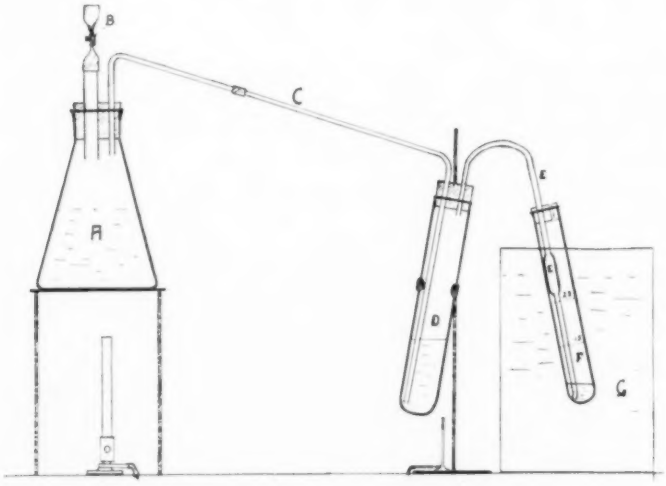

Fig. 1.

should not be inserted in the apparatus until all other parts of the apparatus are in readiness for immediate distillation. The water in *A* should have been brought to boiling previously, with the pinch clamp *B open*. By adjusting the pinch clamp on B, send the desired amount of steam over into *D* until the liquid in *D* is caused to bubble. A microburner may be used to hasten boiling in *D*, and may then be placed in such a way as to warm the sides of tube *D*, thus lessening condensation of steam. When the liquid in *F* has risen to the 15 c.c. mark, slip off the receiving tube *F* from the rubber stopper, and let it rest in a slanting position while the distillation is continued for one minute more. Rinse the lower end of the delivery tube with a little water, cool the distillate and dilute to about 20 c.c. Transfer 0.3 mg. N to a 100 c.c. volumetric flask, dilute to about 75 c.c. Add 10 c.c. Nessler's to standard and 2.5 c.c. to unknown. Make to volume and read in colorimeter with standard set at 20 mm.

Precautions.—All rubber stoppers and connections must be boiled to remove NH_3 before using.

Prior to commencing a series of determinations the water in the flask *A* should be boiled for ten minutes. A portion of the steam emitted should be tested for ammonia by direct Nesslerization. This precaution renders unnecessary the use of nitrogen free water which was originally used in this method.

Water in flask *A* must be kept boiling at an even rate throughout a determination. The use of a few glass beads in the bottom of the flask is desirable to prevent bumping.

In using a microburner to heat the tube *D*, care must be exercised not to heat too vigorously. The rate of admission of steam from *A* must be sufficiently rapid to prevent sucking back of the distillate in *F*. Once an even, moderately fast boiling has been established in the flask and the distilling tube, there is no necessity of watching the distillation continuously.

The apparatus is washed by steam before use by placing an ignition tube containing a little boiling water in place of *D*.

TABLE I

A COMPARISON OF THE STEAM DISTILLATION METHOD WITH THE ORIGINAL FOLIN-WU METHOD
(Values in mg. of Urea Nitrogen per 100 ml. Blood)

NO.	FOLIN-WU	STEAM DISTILLATION
1	10.78	10.80
2	13.60	13.60
3	15.38	15.34
4	18.90	18.80
5	(15.9)*	(15.80)*
	(16.1)	(16.00)
6	(13.3)*	(13.80)*
	(13.3)	(14.10)
7	(14.20)*	(14.40)*
	(14.50)	(14.30)
8	(11.50)*	(11.90)*
	(11.70)	(12.10)

*Duplicate determinations.

RESULTS

Table I presents data comparing the results obtained by this modification with those of the original Folin-Wu method.

SUMMARY

1. The Folin-Wu distillation procedure for blood urea is modified by the inclusion of a steam distillation apparatus.

2. The values obtained by the method are in close agreement with those obtained by the original Folin-Wu procedure.

3. The modification has the advantages of freedom from close technical supervision and eliminates most of the hazards of the original procedure.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

POISONING, Gasoline and Kerosene, in Children, Nunn, J. A., and Martin, F. M. J. A. M. A. 103: 472, 1934.

There appears to be no specific antidote for gasoline or kerosene poisoning. Treatment consists of removing as much of the offending agent as possible by gastric lavage or emesis and laxatives. During gastric lavage there is much retching, struggling and breath catching, which seem to favor aspiration of stomach contents into the lungs. It is the authors' impression that there is less likelihood of aspiration of the fluid when emesis is produced by the oral administration of syrup of ipecac than when gastric lavage is employed. In their more recent cases when cyanosis and other signs of respiratory embarrassment are present, they believe that the use of oxygen (95 per cent) and carbon dioxide (5 per cent) has been of considerable benefit. They therefore recommend the administration of oxygen and carbon dioxide in all cases that show such signs. It is employed for its stimulating action on the respiratory center rather than to increase the oxygen-carrying power of the blood. They have also given atropine sulphate and caffeine sodiobenzoate hypodermically for their stimulating effects.

DYSENTERY, Sonne, Felsen J., and Osofsky, A. G. J. A. M. A. 103: 966, 1934.

Sonne dysentery chiefly affects children and is characterized by a short incubation period, brief course and general mildness.

The disease exists in endemic and epidemic form in the United States.

With increasing incidence it is expected that the general character of the disease will change and, in epidemic form, affect more adults and cause a higher degree of mortality in children.

Sonne dysentery is a contact infection and outbreaks can be terminated by proper epidemiologic measures.

The effects of high titer serums in patients and of specific vaccines in contact cases are now being studied.

SEPTICEMIA: A Method of Treatment, Cadham, F. Am. J. M. Sc. 188: 542, 1934.

A method is described for the treatment of septicemia by inoculation of a specific antiserum and by transfusion of normal human blood serum. A brief report of 100 cases is submitted: 85 of the patients recovered. Excluding the 5 cases of subacute bacterial endocarditis, all fatal, the mortality rate of the other 95 cases was 10.5 per cent.

Numerous strains of streptococci and staphylococci are recovered from patients with septicemia or from patients with pyemic foci resulting from infection with these bacteria. The microorganisms are cultured in serum broth and a vaccine prepared. A number of rabbits are selected and inoculated with a heavy suspension of the vaccine. Vaccine is administered to the rabbit when six months old and the inoculations continued twice weekly throughout the useful life of the rabbit, using numerous strains of streptococci and staphylococci. The subcutaneous and intravenous route are both employed. When one of these animals is two years of age it is considered suitable for the immediate purpose in view. Cadham keeps 25 rabbits continuously immunized.

When a positive culture is obtained from a patient showing symptoms of septicemia, a vaccine of the microorganism is prepared as rapidly as possible (generally within twenty-four hours), and this vaccine is inoculated into a series of the immunized rabbits. On the

following day, from 6 to 10 c.c. of blood are withdrawn by means of a vacuum tube from the heart of one of these rabbits, the blood is allowed to clot, the serum is removed, tested for sterility, and the patient is then inoculated subcutaneously or intramuscularly with this serum. Inoculations with the vaccine of the causative organism are continued daily with the series of rabbits already immunized and which have been selected for use in the particular case under treatment. If necessary the serum from the blood of these rabbits in rotation is administered to the patient on successive days.

The patient's blood is directly matched for a compatible donor. It is of advantage to have this procedure completed on the earliest indication that the case may prove to be one of septicemia. The suitable donor reports to the laboratory and from 60 to 100 c.c. of blood are withdrawn into a large vacuum tube. The blood is allowed to clot at room temperature, and subsequently placed in the ice chest for twelve to eighteen hours. The serum is removed and the patient is transfused with this serum. If considered necessary, this procedure is also carried out daily, in which case it may be advisable to have more than one donor. As a rule Cadham has administered the human serum on alternate days. The average number of combined treatments administered to the patients was 4, the greatest number administered to any single patient, 10.

No drugs were given during the course of the treatment. On occasion, whisky was prescribed as a stimulant and then sparingly. The patient was encouraged to partake of a fairly liberal diet.

Serum treatment was instituted on the average on the sixth day following the initial symptoms; the earlier administration was on the third day, the most delayed on the sixteenth day. The first treatment with serum administered to a patient with subacute bacterial endocarditis was six weeks subsequent to the initial symptoms.

PREGNANCY, Conservative Treatment of Late Toxemias of, Mc Neile, L. G. J. A. M. A. **103:** 548, 1934.

In 799 cases of late toxemias of pregnancy, 259 were of the eclamptic type.

There was a definite decrease in the mortality in eclamptic patients treated by conservative methods at the Los Angeles County General Hospital.

The intravenous injection of a 10 per cent solution of magnesium sulphate has proved a very valuable adjunct in the treatment of the late toxemias of pregnancy. It will cause some reduction of blood pressure, reduce edema, increase urinary output and reduce or control other symptoms.

The intravenous injection of a 10 per cent solution of magnesium sulphate will control the convulsions of eclampsia in nearly every case, and exercises a favorable influence on the other symptoms of eclampsia.

The intravenous injection of a 10 per cent solution of magnesium sulphate is clinically proved to be a safe procedure.

ANEMIA, Classification and Treatment of, on the Basis of Differences in the Average Volume and Hemoglobin Content of the Red Corpuscles, Wintrobe, M. M. Arch. Int. Med. **54:** 256, 1934.

Observations on the volume and hemoglobin content of the erythrocytes of more than 1,000 healthy and anemic persons are recorded and discussed under the heads of accuracy of the methods employed, normal values, physiologic variations and variations in disease.

The anemias may, on the basis of differences in the mean volume and hemoglobin content of the red corpuscles, be subdivided into four groups. Each of these groups appears to correspond to a fundamentally different type of disturbance in the hematopoietic apparatus. (1) Macrocytic, (2) Normocytic, (3) Simple microcytic, (4) Hypochromic microcytic.

Consequently the method of classification proposed is useful as an aid to diagnosis. It facilitates also the separation of conditions in which liver therapy may be expected

to be of value from those in which treatment with iron preparations is successful, and distinguishes these two types of anemia from those in which neither method of treatment can be expected to succeed.

JAUNDICE, Hemolytic, Mechanism of Increased Fragility of Erythrocytes in, Haden, R. L. Am. J. M. Sc. **188**: 441, 1934.

The erythrocytes in congenital hemolytic jaundice constantly exhibit spherocytosis, best shown by the increased volume-thickness index.

The diameter of the red cell in this condition is always less than normal; the volume is variable.

When placed in hypotonic salt solution, normal erythrocytes become progressively more globular with little change in diameter as the solution is made more hypotonic.

There is a direct relation between the volume-thickness index and the fragility of red cells.

In congenital hemolytic jaundice, the erythrocytes have at the beginning one of the shapes through which a normal cell must pass when placed in successive dilutions of hypotonic salt solution, and so may be regarded as nearer the hemolysis point.

The one fundamental variation from normal in congenital hemolytic icterus is the microspherocytosis. The anemia, jaundice, splenomegaly, reticulocytosis and increased fragility are all secondary to the globular form of the erythrocyte.

AUTOHEMAGGLUTINATION, Sherman, I. Am. J. M. Sc. **188**: 487, 1934.

Three cases are reported in which autoagglutination of the red blood cells occurred at room temperature and disappeared at body temperature.

The phenomenon is due to the presence of agglutinins in the serum which produce clumping of red blood cells of all four blood groups at temperature below a critical point. This point is between 30° and 31.5° C.

Two of the cases reported belong to Group 0 and 1 to Group A.

All 3 cases were suffering with a febrile infectious condition. Reexamination in one case, after recovery of the patient, showed diminution of the agglutinizing power of the serum to an extent that the clumping of the red cells occurred only in the ice box but not any longer at room temperature.

Two of the 3 patients received blood transfusions 5 and 2 times respectively, without any untoward reaction.

ANEMIA, Pernicious, Relation Between Total Reticulocyte Count and the Degree of Bone Marrow Involvement, Bethell, F. H. Am. J. M. Sc. **188**: 476, 1934.

In an effort to estimate the comparative quantities of reticulocytes produced during the initiation of a remission in different pernicious anemia patients, the areas circumscribed by the graphs of the "reticulocyte responses" plotted on a standard paper, were measured in square centimeters.

The value so obtained for any patient is an expression of the total reticulocyte response of that patient.

From the data obtained, formulas were constructed by which a measure of the expected total reticulocyte response can be secured from a knowledge of the initial red blood cell count.

For liver extract by mouth $R_r = \frac{67.37}{\frac{0.369}{Eo}}$ sq. cm.

For desiccated stomach $R_r = \frac{86.93}{\frac{0.385}{Eo}}$ sq. cm.

In which R_r is the "area of reticulocyte response" in square centimeters and Eo the initial erythrocyte count (millions per c.mm.).

By the use of these formulas curves were prepared for the "area of the reticulocyte response" similar to those devised by Riddle and by Bethell and Goldhamer for the maximum reticulocyte percentage response, in terms of the initial red blood cell level.

HYPOGLYCEMIA, In Diabetes Associated With Obstruction of Pancreatic Duct, Zeckwer, I. T. Arch. Int. Med. **188**: 330, 1934.

A patient with a history of diabetes but no medication with insulin manifested hypoglycemia. At autopsy, calculi obstructed the pancreatic duct, with resultant extraordinary atrophy of the acini and islands; the liver showed degenerative changes, and there was pulmonary tuberculosis.

The clinical condition paralleled experimental pancreatectomy in the dog, in which it has been shown that hepatic insufficiency results from deprivation of the external secretions of the pancreas.

Points of similarity between the clinical case and the experimental animal were: (1) the development of hypoglycemia after the hyperglycemic period and (2) hepatic degeneration. The latter, however, was of a minor degree and of a different gross and histologic character from that seen in dogs.

The factor of undernutrition must be considered in the production of the hypoglycemia in this case, but has less experimental basis than the effect of loss of external pancreatic secretions.

In 10,300 autopsies performed in this department, only 4 other instances of pancreatic calculi could be found, none of which was associated with diabetes.

It is suggested that when the liver is damaged through loss of the external secretions of the pancreas, especially if the condition is aggravated by undernutrition and tuberculosis, hypoglycemia may result in diabetes even in the absence of treatment with insulin.

INFLUENZA, Epidemic, A Note on the Suggested Epidemiological Relationship of Certain Strains of Green Streptococci to, Noble, W. C., and Brainard, D. H. Am. J. Hyg. **20**: 191, 1934.

Several workers have reported the isolation of specific strains of green streptococci from the throats and blood of influenza patients. Falk claims that he has repeatedly isolated strains identical with his 42xS and 18dR from human influenza material, and from monkeys inoculated with this material. The authors have studied the agglutination of a large number of strains which they have isolated from influenza patients and from other sources. None of these strains is agglutinated by the antiserum for 18dR. Some of the strains cross-agglutinate in unabsorbed serum with 42xS; they may therefore bear group relationship to 42xS, but they are certainly not identical with it. It should be noted that they were isolated as frequently from noninfluenzal as from influenzal sources, three of them being isolated from normal, uninoculated monkeys.

Rosenow and Falk state that they have found agglutinins for their strains in the serum of influenza patients. The serum from none of our influenza patients or convalescents contained agglutinins for either 42xS or 18dR.

The authors have therefore found no evidence that either of these organisms is the cause of influenza.

AGRANULOCYTIC ANGINA, Following Ingestion of Dinitrophenol, Bohn, S. S. J. A. M. A. **103**: 249, 1934.

Agranulocytic angina developed in a patient after the ingestion of 21.8 gm. of 2, 4 dinitrophenol sodium over a period of four months.

The dosage was 4 mg. per kilogram of body weight daily for two weeks and then doubled until the onset of unfavorable reactions.

Treatment consisted of discontinuing the drug, administration of pentnucleotide and one transfusion of 250 c.c. of whole blood, following which the patient recovered.

TUBERCULIN, Skin Sensitiveness to, in Primary Tuberculosis, Stewart, C. A. J. A. M. A. 103: 175, 1934.

The average degree of skin sensitiveness to old tuberculin present in a group of infected children with negative or inconclusive chest films was essentially equal to that present in groups of cases with definite and conspicuous demonstrable intrathoracic lesions in different stages of resolution and considered characteristic and typical of tuberculosis of first infection. The mean level of allergy to tuberculoprotein produced in groups of children by chance infections is independent, therefore, of the absence, presence, scarcity, abundance, size or character of the primary tuberculous lesions demonstrable by roentgen examinations during life.

The distribution of different degrees of skin sensitiveness to old tuberculin in a group of infected children consisting of cases with normal or inconclusive chest films was found not to differ essentially from that present in other cases with distinct demonstrable intrathoracic lesions deemed characteristic of tuberculosis of first infection. The curves that represent the general pattern of the distribution of different degrees of allergy to tuberculoprotein found in each group of cases are very similar.

This study on living patients failed, therefore, to disclose evidence that justifies one in entertaining suspicions that children with large and intense Mantoux reactions harbor more extensive pathologic changes or have more active primary tuberculous lesions than do other children who are less sensitive to tuberculin.

Groups of children with primary tuberculosis exclusively, who are dissimilar roentgenologically, are alike as far as the average group skin sensitiveness to tuberculin is concerned. Apparently soon after tubercle bacilli make their initial entry into the body and before the primary tuberculous lesions have time to resolve, allergy reaches an average group level that is sustained with little or no definite change throughout the first few years that postdate the primary infection.

Changes in the size of Mantoux tests throughout the periods of increment and decline of the reactions in children with normal or inconclusive chest films roughly parallel similar changes manifested by skin reactions in other children with definite demonstrable intrathoracic lesions typical of tuberculosis of first infection.

TUBERCULIN TEST, The Intracutaneous Quantitative in the Diagnosis of Active Tuberculosis, Ayman, D. J. A. M. A. 103: 154, 1934.

It seems clear that with a given batch of old tuberculin it is easily possible to determine a dilution of this which will give a minimum of positive reactions of a certain size in patients without active tuberculosis and a maximum of such positive reactions in patients with active tuberculosis. More specifically, in the present study a 1:50,000 dilution of old tuberculin injected into 202 nontuberculous patients was found to give 97 per cent negative results; i.e., reactions less than 10 by 9 mm. in size. The same dilution injected into 82 patients with active tuberculosis gave 69 per cent of positive reactions 10 by 9 mm. in size, and when severely ill patients with high fever and bronchopneumonic areas in the lungs were subtracted from the total tuberculous group, 93 per cent of the tuberculous patients gave adequate positive reactions 10 by 9 mm. or more in size. In seriously ill patients it is known that the allergic responses are diminished or absent, and since all these patients had every symptom and sign of active tuberculosis, their diagnosis is obvious without the dilute tuberculin test. It therefore seems valid to subtract these seriously ill patients from the total group. Also, the fact that such dilute reactions occur chiefly when the patient is only mildly or moderately ill rather than moribund would appear to enhance the value of the test, for the difficulty in the diagnosis of tuberculosis is greatest in those patients who are not yet seriously ill.

With only 3 per cent of clinically nontuberculous patients reacting to the 1:50,000 dilution with areas 10 by 9 mm. or more, and from 69 to 93 per cent of such positive reactions in patients with active tuberculosis (depending on whether one includes seriously ill and moribund patients), Ayman believes that such a test has definite diagnostic value.

This is in complete agreement with the previous studies. Since it is a specific test, unlike the nonspecific white blood cell count or fever and pulse chart, it would seem to merit as much as these methods in the diagnosis of active tuberculosis. It is very unlikely that such weak dilutions will produce other than skin reactions. Ayman has not encountered a single focal or general reaction after the 1:50,000 dilution.

With the recent availability of the crystalline tuberculoprotein, it should soon be simple to determine once for all a dilution of this tuberculoprotein which will give these reactions. Then such a dilution will always be the one to use, whereas at present the proper dilution of the old tuberculin will have to be determined for each batch.

BACTERIAL CARBOHYDRATES, Serological Specificity, With Special Reference to Type II Pneumococcus and a Heterophile Strain of Bacterium Lepisepticum, Dingle, J. H. Am. J. Hyg. **20**: 148, 1934.

Specific carbohydrate substances have been isolated from Types I, III and III *Diplo. pneumoniae, Past. cuniculicida, Sacch. cerevisiae, E. coli,* and *P. marginalis,* and the method of preparation has been outlined.

The carbohydrate substances so isolated have been found to react specifically in high dilution with homologous immune sera and to be free from protein as evidenced by the biuret reaction.

The carbohydrate substances of *Past. cuniculicida* and *P. marginalis* isolated from these organisms for the first time, were found to be soluble in aqueous or saline solution, to react with homologous antisera in high dilution, to give positive Molisch reactions and negative biuret tests, and to be nitrogen-free. They did not reduce Benedict's solution nor did they give a color with iodine.

Determinations of the specificity of the bacterial carbohydrates were made by carrying out precipitin reactions with solutions of the carbohydrate substances and antisera against a variety of bacteria.

The cross-reacting precipitations occurring with the polysaccharides from Types I, III, and III pneumococcus and antisera against various bacteria were for the most part slight.

A pronounced characteristic carbohydrate precipitate, however, was obtained with rabbit antisera for the heterophile *Past. cuniculicida* 370 with Type II pneumococcus carbohydrate. Slight cross-reactions were also noted between the *Past. cuniculicida* carbohydrate and both Type II antipneumococcus rabbit serum and Type III antipneumococcus horse serum.

The polysaccharide from *Sacch. cerevisiae* did not give cross-reactions with Type II antipneumococcus serum as had been reported by Sugg and Neill, but did give slight reactions with antisera against *P. marginalis,* Friedlander's bacillus, and *Past. cuniculicida* 370.

Reactions of almost uniform intensity were noted between the carbohydrate from *Sacch. cerevisiae* and rabbit immune sera against the seeds of lettuce, squash, beet, wheat, cabbage, sunflower, alfalfa, carrots, corn, peas, and beans.

Some variation in the intensity of the reactions of *Past. cuniculicida* 370 carbohydrate with homologous sera from different rabbits was noted, but no reaction at all was given with the antiserum for the avirulent strain, RD3.

The carbohydrate isolated from a fecal, encapsulated strain of *E. coli* reacted specifically with its homologous antiserum but with no others. It did not react with an antiserum for a strain of nonfecal *E. coli.*

The carbohydrate from *P. marginalis* showed slight reactions with Types II and III antipneumococcus horse sera, and corn and wheat antisera. In determining the group specificity of this substance, it was found that three of the seven antisera for organisms of the genus Phytomonas showed slight reactions: *P. marginata,* and the hop and daisy strains of *P. tumefaciens.*

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

The Biochemistry of Medicine*

THAT a second edition should become necessary in two years bears out the prediction made in a review of the first edition that this book would be favorably received by the practitioner as well as by workers in special fields.

The present volume represents the changes necessary to bring the text in line with the results of newer studies.

The section on endocrines has been rewritten and now includes an account of anti-endocrine compounds. Changes are found also in the section on vitamins, and, in fact, practically all the various chapters have been revised and often extended. New additions include Von Gierke's glycogen disease, diseases related to disturbances of lipoid storage, dinitrophenol in obesity, glycine treatment of myasthenia gravis, and the pentose-nucleotide treatment of agranulocytosis.

Diseases of the Mouth and Their Treatment†

THAT the mouth and its contents present varied changes in disease, changes sometimes of differential value and even of pathognomonic import, has long been recognized, while its importance as a source of focal infection has been widely discussed in medical literature. In view of these facts the present volume by Prinz and Greenbaum should be of interest to practically all who are engaged in the study of disease.

The specific object of this book is stated in the preface: to gather the enormous mass of scattered information on the subject and present it in an orderly and systematic manner to the medical and dental professions. That this task has been carefully and comprehensively carried out the table of contents bears witness.

As a reference this book should prove of great value to the allied professions of medicine and dentistry as suggesting the importance of a careful survey of the mouth, not only in the presence of oral disease but of disease in general.

The section on gingivitis should be of great value for while this is, perhaps, the most commonly encountered disease in the mouth, it is nevertheless one concerning which the general information is most likely to be confused.

The reviewer, therefore, welcomes its clean-cut and logical discussion in this book. Concerning the constant and varied bacterial flora of the mouth even in health, and recognizing that most of these organisms are peculiarly opportunists, it is apparent that, whatever the primary cause of gingivitis may be, the clinical and laboratory picture is almost immediately complicated, and not infrequently to no small extent determined, by the secondary bacterial invasion.

The borderline between one kind of gingivitis and another is hence often nebulous. Particularly is it refreshing to see that the gingivitis characterized by a *predominance* of Vincent's organisms, the "trench mouth" of the laity, is clearly stated to be a condition subject to *arrest* rather than cure. For those familiar with its bacteriology have long realized that successive attacks are not new events but represent a recurrence of the original disease.

It is to be expected, of course, that the first edition of any book of this kind is subject to minor criticisms.

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*The Biochemistry of Medicine. By A. T. Cameron, Professor of Biochemistry, University of Manitoba, and C. R. Gilmour, Professor of Medicine, University of Manitoba, Ed. 2, Cloth, pp. 518, 31 illustrations. Wm. Wood & Co., Baltimore.

†Diseases of the Mouth and Their Treatment. By Herman Prinz, Professor of Materia Medica and Therapeutics, School of Dentistry, University of Pennsylvania, and Sigmund S. Greenbaum, Associate Professor of Dermatology and Syphilology, Graduate School of Medicine, University of Pennsylvania. Cloth, 602 pages, 287 engravings, 11 colored plates. Lea and Febiger, Philadelphia, Pa.

The section on the preparation and examination of smears from the mouth—referred to in the book as "Microscopic Examination of the Oral Cavity," is, it seems to this reviewer, somewhat sketchy. The excellent work of Keilty in this field in which these methods are well worked out, is not referred to; the Weiss stain for Vincent's organisms might well be included; the method of staining tubercle bacilli in the cold has long supplanted the use of heat; and, while *B. leprae* is acid-fast, decolorization must be carried out with more care than when staining for tubercle bacilli.

Incidentally, the book is not restricted to a consideration of diseases of the mouth per se but includes also a discussion of the changes seen in the mouth in disease in general thus rounding off the subject.

This book can be recommended as a useful contribution. The numerous illustrations are excellent and helpful.

Handbook of Anesthetics*

THE fourth edition of this well-known and excellent little book has been thoroughly revised to include the new methods and new apparatus which have established a definite claim to recognition.

As before, this book remains an authoritative and comprehensive presentation of anesthesia and may be regarded as a safe guide to technic.

Recent Advances in Endocrinology†

ENDOCRINOLOGY has come to be accepted as a term indicating the science concerned with the study of internal secretion, by which is meant the products of those glands which separate within themselves specific compounds and secrete them generally into veins, very occasionally into lymph vessels.

Endocrinology is a relatively new development and as such has suffered from a confusion of ideas and a still more marked confusion arising from a more or less haphazard terminology. As Cushing has aptly said: "Endocrinology lends itself to two glaring faults, one the popularization of writing on the subject, and the other a tendency of clinical observers to draw upon their fancy in a symptomatology which does not lend itself to precision."

Under these circumstances it is not to be wondered at that the clinician, even though he be interested in the subject, should have difficulty in keeping pace with the increasing studies made and reported in this field, and still more difficulty in assigning to each its proper value and importance.

For this reason, the first edition of Professor Cameron's book, presenting as it did a sane, comprehensive, and careful review of this difficult subject, was a welcome addition to the medical library.

Equally as welcome is this second edition which takes cognizance of and includes the studies in endocrinology up to August, 1934. As a consequence this book has undergone an extensive revision. Important revisions have been made upon varied subjects, hyperparathyroidism, hypoglycemia, diabetic treatment, the replacement therapy of ovarian disorders, and studies on the pituitary.

The book contains, in addition to an introductory chapter, discussions on: The Thyroid Gland and Iodine Metabolism, The Parathyroid Glands, The Islets of Langerhans and Insulin, The Adrenal Glands, The Endocrine Secretions of the Organs Concerned With Reproduction, The Pituitary Gland, Some Active and Presumptive Endocrine Principles, and finally a discussion on Endocrine Interrelationships and Antagonisms.

The general utility of the book is enhanced by a good index.

This volume can be commended to the practitioner in search of practical and usable information.

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*Handbook of Anesthetics. By J. Stuart Ross, Late Lecturer on Practical Anesthetics, University of Edinburgh, and H. P. Fairlie, Anesthetist to the Western Infirmary, Glasgow, etc. With a Chapter on Local Anesthesia by W. Quarry Wood, Assistant Surgeon, Edinburgh Royal Infirmary. Cloth, ed. 4, pp. 299, 65 illustrations. Wm. Wood & Co., Baltimore.

†Recent Advances in Endocrinology. By A. T. Cameron, M.A., D.Sc. Professor of Biochemistry, University of Manitoba. Cloth, ed. 2, pp. 406, 55 figures, P. Blakiston's Son & Co., Philadelphia, Pa.

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EDITORIALS

John James Rickard Macleod

MACLEOD, the eminent physiologist, co-discoverer of insulin, passed away at his home in Aberdeen, Scotland, Saturday, March 16. For more than two years he had been afflicted with an arthritic infection, but kept on with his labors—teaching and writing—betraying by neither word nor sign of any kind that he believed the end to be near. He carried with him to the last the tradition of his race—to do one's best under all conditions and never complain.

Macleod was born at the manse in Cluny, near Dunkeld, Scotland, September 6, 1876, the son of the late Rev. Robert Macleod. He was graduated from Marischal College, University of Aberdeen, with the degrees of M.B., Ch.B., in 1898, and four years later obtained the degree of D.P.H. from Cambridge University. About ten years ago the honorary degree of LL.D. was bestowed upon him by University of Aberdeen. In 1903, he was made Professor of Physiology at Western Reserve University, and held this chair until 1918 when he accepted

the Chair of Physiology at the University of Toronto. He remained with this University until 1928, at which time he accepted the Chair of Physiology at Aberdeen University, succeeding Prof. J. A. MacWilliam, who had resigned. In 1922, while connected with the University of Toronto, his collaboration and helpful enthusiasm brought success to the labors of Banting and Best, who succeeded in producing from the pancreas a secretion which they named "insulin," and proved its therapeutic value in the treatment of diabetes. For this discovery these three won immediate recognition throughout the scientific world, and Macleod and Banting were awarded the Nobel prize in medicine for 1923.

It was my good fortune to have been closely associated with Macleod for nearly twenty years. Through a chance meeting with his assistant, Roy G. Pearce, in February, 1915, this acquaintance began, and from it ultimately came Macleod's important contribution to physiology, *Physiology and Biochemistry in Modern Medicine*, the first edition published in 1918. His enthusiasm and clear thinking impressed every one with whom he came in contact. His was the genuine scientific mind. Statements and beliefs meant nothing to him. Only positive proof—so positive that nothing but truth remained—was acceptable. In meditating over his life since his passing, I am reminded very much of Livingston. Both Scotch, both much alike in physical appearance, both with a profound courage and consuming enthusiasm, both sons of clergymen, both working at the end of life under great physical handicaps but without a murmur or complaint. All during the summer of 1934, Macleod read galley and page proof on the seventh edition of *Physiology in Modern Medicine*, and never in any way did he betray that he was ill. Less than a month before his passing I had a long letter telling me of his satisfaction with this, his last edition, and of his hopes for future editions.

Early in my contact with him he expressed the feeling that physiology was of value only in its relationship to clinical medicine and surgery. He taught physiology with this constantly in mind; a viewpoint that is accepted and proclaimed everywhere today.

When the *Journal of Laboratory and Clinical Medicine* was started in 1915, Macleod was a member of the Advisory Editorial Board. With him were Roger S. Morris, Paul G. Woolley, Roy G. Pearce, Dennis E. Jackson, F. P. Gay, Hans Zinsser. Victor C. Vaughan was Editor-in-Chief. Now only Jackson remains of this original group. Gay, Zinsser and Pearce resigned after a few years; Vaughan, Morris, Woolley, Macleod are gone. It was through the pages of this JOURNAL that the discovery of insulin was first published in a medical journal.

It has been the good fortune of few men in medicine to bestow upon mankind a gift so great and so lasting as was bestowed upon it by Macleod in the help he rendered in the discovery of insulin. Vaughan, in commenting on this to me soon after its proof was definite, ranked it of equal importance with the work of Jenner, Pasteur, von Behring and Koch. In fact, he pronounced it of equal value with any discovery ever made in medicine, and so it has proved. Few indeed are the men in medicine who live to see the great work they do bless and benefit mankind. It was Macleod's good fortune to see this, and

seeing it, no doubt, made death easier for him. With one's work done, especially a work so beneficial as that done by Macleod, one can face the end just as one lies down at the close of the day to sleep peacefully, knowing that he has done his best.

His students caught the master's vision and enthusiasm. He was never so happy as when he had a group around him, demonstrating, proving, inspiring. To these he has passed the torch, and in them will his life and his work live on, while the millions now and in the future who live because of insulin bless and revere his name.

—*C. V. Mosby.*

Glycine, Glycocoll, or Amino-Acetic Acid

GIBSON and Martin and Brand, Harris, Sandberg and Ringer, in their investigations of the origin of creatine, were among the first to recognize the importance of glycine. The significant relationship between glycine,* a substance that the normal person can readily synthesize, and creatine, which has been demonstrated to play an important part in muscle function, suggested to several investigators that prolonged administration of the amino-acid might influence the myopathies. The discovery of metabolic functions of creatine in muscle metabolism has attracted considerable clinical interest in the study and treatment of disorders of the muscular system. Evidence is gradually accumulating which indicates that the metabolism of creatine has a wider clinical importance than in the myopathies alone, for changes in creatine excretion have been noted in individuals not suffering from disorders of the muscular system.

Karl Thomas, one of the most noted of German physiologic chemists, has been particularly interested in all problems connected with metabolism. From his laboratory in Leipzig came a report of the beneficial effect of the administration of glycine to patients with progressive muscular dystrophy. This was a rather striking confirmation of the hypothesis that glycine might influence the myopathies.

Following this work of Thomas, Milhorat and Technor, Boothby, in conjunction with Wilder, Hench and the section of neurology, of the Mayo Clinic, not only studied the use of glycine in muscular dystrophy but also extended the investigation to include patients with undoubted myasthenia gravis and even cases of variations of fatigability unassociated with any type of muscular pathologic changes.

Since these early reports Remen, Schmitt, Van Dalsem, Reese, Taylor, Beard, Tripoli, and others have used glycine in cases of myasthenia gravis and feel confident that glycine as a therapeutic agent in this condition is of significant value. Although in a very few of the most severe cases benefit from it has not been obtained, unmistakable benefit has been obtained in other severe cases and in all of the milder cases. The improvement obtained is not only in the increased

*Glycine must not be confused with the photographic developer sold under the trade name, glycin, which is an entirely different substance and is distinctly poisonous.

strength of the patients but also in their gains in weight and improved tonus of their muscles.

Beneficial results have been substantiated in muscular dystrophy by Kostakow and Slauck, Chanutin, Britt and Royster, Beard and Tripoli, although it would appear that the treatment of muscular dystrophy has not been successful in many cases and certain cases do not respond at all.

No ill effects from the use of glycine have been noted. Kidney function even after long periods of glycine administration as measured by urea clearance has remained unchanged.

Glycine which is the simplest of the series of amino-acids is known to be synthesized by the body. Glycine is present in small amounts in many foods; gelatin, however, is the only common substance that contains large amounts of it. It is not considered as an essential amino-acid, in other words animals can live without any in their food, probably because the body under normal conditions can make all it needs. As a result little attention has been given to the possible necessity or even the advisability of administration of glycine.

Glycine should be considered not as a drug but rather a food, in fact as one of the building stones of tissue. At present not much of anything is known of the actual chemical processes involved in the intermediary metabolism. It is possible that even though our bodies synthesize glycine, under certain abnormal conditions the synthesis may be inadequate to prevent muscular fatigability.

Recent investigations of the use of this amino-acid treatment have aroused great interest and have opened a new chapter in the field of metabolism. Glycine offers hope in the treatment of certain myopathies, adds metabolic evidence of the differences existing among these various muscle diseases, and creates a new viewpoint to attempt to elucidate the function and importance of creatine and creatine precursors in muscle physiology.

Early mild stages of myasthenia gravis may be frequently overlooked and mistaken for chronic nervous exhaustion. A diagnosis may be given of functional, neurotic or hysterical and the true condition recognized only after the progress of the disease has developed definite objective signs. The clinical study of patients whose main complaint is fatigability requires in a differential diagnostic effort the consideration of the possibility that these symptoms may be indicative of an early or mild transient form of myasthenia gravis. Oppenheim's original description of the cardinal symptoms of myasthenia gravis, namely weakness and fatigability of groups of muscles, especially those innervated by cranial nerves (bulbar palsy, ptosis, ophthalmoplegia) should be borne in mind.

However, a word of caution should be given in the application of glycine to various fatigabilities of patients without pathologic changes in muscle. Although it will be of interest to observe the effects of glycine in simple fatigability great care in the subjective interpretation of symptomatic results will be necessary.

The studies and recent clinical investigations of the use of glycine are indeed very enlightening and open a new field of metabolic study. Glycine, however, will probably not prove to be a panacea for all disturbances of muscles.

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—*C. J. B.*

CORRESPONDENCE

To the Editor:

IN REGARD to Dr. Kracke's letter in your journal for January, in which he criticized my article on the rôle of amidopyrine in the etiology of granulocytopenia, I feel that several of his criticisms are entirely unwarranted, and contrary to the consensus of scientific opinion.

Dr. Kracke states that amidopyrine is prepared from phenyl hydrazine and comes ultimately from benzene and anilin, and goes on to state "it is not prepared from pyrazolon." Quite true, but can he deny that, regardless of its derivation, it contains a pyrazolon ring?

Dr. Kracke states that amidopyrine does not have an imido radicle, but that it has an amine radicle instead. In this statement he is absolutely wrong, as shown by McGuigan, who in *A Textbook of Pharmacology and Therapeutics* (Saunders, 1928), page 137, states: "The antipyretic action apparently resides in the benzene ring. Benzol itself is not antipyretic because it cannot enter into reaction with the body tissues, but phenol has some antipyretic action, anilin and phenyl hydrazine act more strongly. Paraminophenol is also antipyretic. The latter is more antipyretic and causes less blood destruction than anilin or phenyl hydrazine. In antipyrine, the pyrazole ring or complex apparently is also antipyretic since the antipyretic properties are absent in 1-phenyl 3-methyl pyrazolon, and only when the *imido* hydrogen is replaced by methyl does it appear." Naturally what is true of antipyrine, is equally true of its derivative, amidopyrine.

In *An Introduction to Chemical Pharmacology* (Blakiston, 1921), McGuigan classes antipyrine as an artificial alkaloid, and states that like alkaloids, it unites with acids, and when prepared, it is combined with HI. In his list of alkaloids, on page 224, he includes antipyrine.

Kracke states that there is insufficient evidence to ban amidopyrine. Yet, in his article on "The Etiology of Granulopenia" (*American Journal of Clinical Pathology* 4: 453, 1934), he states: "first, that granulopenia, based on a review of 1,385 death reports, is a disease that affects nurses, physicians and medical people more than any group in the country; second, that certain drugs of the benzene ring class are capable of being oxidized to toxic products which are capable of producing granulopenia in rabbits." Further in the same article, he states: "It seems that there is constantly increasing evidence that acute fulminant granulopenia and probably the chronic types as well are caused for the most part by the administration of easily oxidizable benzene ring drugs. In the literature there now have accumulated reports of fifty cases in which these drugs are incriminated; *notably amidopyrine,* occasionally phenacetine and in one instance dinitrophenol." Furthermore, the Food and Drug Division of the U. S. Department of Agriculture has recently sent out a bulletin warning physicians about two dangerous drugs—amidopyrine and cinchophen. The burden of proof is now upon the champions of amidopyrine.

When Kracke classed amidopyrine as a benzene ring compound, I saw fit to disagree with him in my original article. I still maintain that it is a pyrazolon compound, and that the benzene is a side chain. I have already quoted McGuigan in classifying a pyrazolon compound, antipyrine, as an artificial alkaloid. Evidently, he regards it as entirely different in structure from acetanilid and phenacetin, both of which are mentioned in his book, but neither of which is considered an alkaloid. Furthermore, Beckman in *Treatment in General Practice* (Saunders, 1934), under "Agranulocytosis—Therapy" gives the heading: "The Withholding of Drugs Containing Pyrazolon Derivatives," and mentions specifically Madison and Squier's 14 cases, stating that all of these gave a history of having taken *amidopyrine.* Evidently, he recognizes amidopyrine as a *pyrazolon* compound.

As to the safety of acetanilid, which Kracke seems to doubt, in Kracke's own article, previously quoted, on page 455, he states: "We reported one patient who took large amounts of acetanilid. However, this patient has since been reinvestigated and was found to have

received amidopyrine also. We believe, therefore, that acetanilid can be ruled out as an etiologic drug since there is no report of any case of granulopenia following the use of that drug alone.''

As to the advisability of combining acetanilid with alkalies and caffeine, Worth Hale demonstrated that alkalies decreased the toxicity of acetanilid by 20 to 25 per cent, but thought that caffeine increased the toxicity. McGuigan, during the past year, exploded this theory in regard to caffeine, and by carefully conducted experiments, showed that caffeine reduced the toxicity of acetanilid. Furthermore, the U.S.P. preparation, pulvis acetanilid compound, consists of acetanilid, caffeine, and sodium bicarbonate in the proportions of 100, 15, and 30.

The *J. A. M. A.* under the editorial column of *Therapeutics* (**104**: 1229, 1935), under ''Analgesia,'' recommends aspirin for the milder types of pain, and states that if it does not suffice to relieve the pain, acetanilid may be added to it. It also refers to specific indications for phenobarbital, morphine or codeine. It ignores amidopyrine.

I still maintain that the coal-tar analgesics should be placed into three distinct groupings: (1) acetanilid and phenacetin, derivatives of anilin, and excreted as paraminophenol; (2) antipyrine and amidopyrine, pyrazolon compounds, excreted largely unchanged; and (3) the salicylates, excreted as ethereal sulphates and glycuronates. I do not regard it as chemically or medically correct to include groups (1) and (2) together as ''benzamide compounds'' or benzene ring derivatives, as I consider the pyrazolon ring important in differentiating the two, and I consider that they comprise two distinct groups. I also maintain that amidopyrine has been our worst offender as the causative factor of granulocytopenia, and we should recognize this fact, and properly classify the drug as a pyrazolon compound, and not obscure the issue by speaking of ''benzamine drugs,'' and ''benzene ring drugs,'' where the benzene ring may have no bearing upon the case, as the other benzene ring drugs do not have the disastrous effects of amidopyrine. Those who adhere to the ''benzene ring'' theory will have to produce greater evidence than they have thus far produced, to give credence to their theory. Meanwhile, I shall adhere to the hypothesis which clinical evidence seems to produce—amidopyrine is distinctly different from other coal-tar analgesics; and in my opinion, the pyrazolon group, and more specifically the imido radicle, is responsible for its toxic properties.

Very truly yours,

(Signed) LUCIUS FELIX HERZ, PH.B., M.D.

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